CEEHRC NETWORK
Canadian Epigenetics, Environment and Health Research Consortium Network

RÉSEAU CCREES
Consortium canadien de recherche en épigénétique, environnement et santé

The Virtual

7th CANADIAN CONFERENCE ON EPIGENETICS

3-5 November 2021

GenomeCanada

CIHR IRSC

Welcome!

Dear Colleagues,

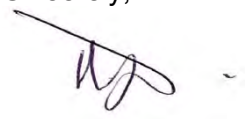
It is a pleasure to welcome you to the 7th Annual Canadian Epigenetics and Environment Health Research Consortium Epigenetic Conference being held virtually.

This year we welcome over 290 national and international epigenetics researchers to share their research and join in discussion. Over the next 3 days we will cover an outstanding scientific program that includes distinguished invited speakers and selected talks covering a breadth of emerging epigenetics research. These topics will be covered over 8 sessions that include keynote and plenary lectures, selected oral presentations, a knowledge translation workshop, and 2 virtual poster sessions. While this year will naturally have a different feel from previous meetings, we hope that the meeting format will encourage interaction and networking across career stages while providing an inclusive 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. During this meeting, we are delighted to announce the launch of a second round of community access to epigenomic mapping capacity through the CEEHRC Mapping Centres at McGill University and the BC Cancer Research Institute and UBC. 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.

Sincerely,



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

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Ottawa Health Research Institute

Marco Gallo
University of Calgary

Martin Hirst
University of British Columbia

Nada Jabado
McGill University

Steven Jones
BC Cancer

Sarah Kimmins
McGill University

Tony Kwan
McGill University

Ariane Lismer
McGill University

Serge McGraw
Université de Montréal

Keynote Speakers

Manolis Kellis
Massachusetts Institute of Technology

Manolis Kellis is a professor of computer science at MIT, a member of the Broad Institute of MIT and Harvard, a principal investigator of the Computer Science and Artificial Intelligence Lab at MIT, and head of the MIT Computational Biology Group (compbio.mit.edu). His research includes disease circuitry, genetics, genomics, epigenomics, coding genes, non-coding RNAs, regulatory genomics, and comparative genomics, applied to Alzheimer's Disease, Obesity, Schizophrenia, Cardiac Disorders, Cancer, and Immune Disorders, and multiple other disorders. He has led several large-scale genomics projects, including the Roadmap Epigenomics project, the ENCODE project, the Genotype Tissue-Expression (GTEx) project, and comparative genomics projects in mammals, flies, and yeasts. He received the US Presidential Early Career Award in Science and Engineering (PECASE) by US President Barack Obama, the Mendel Medal for Outstanding Achievements in Science, the NSF CAREER award, the Alfred P. Sloan Fellowship, the Technology Review TR35 recognition, the AIT Niki Award, and the Sprowls award for the best Ph.D. thesis in computer science at MIT. He has authored over 240 journal publications cited more than 115,000 times. He has obtained more than 20 multi-year grants from the NIH, and his trainees hold faculty positions at Stanford, Harvard, CMU, McGill, Johns Hopkins, UCLA, and other top universities. He lived in Greece and France before moving to the US, and he studied and conducted research at MIT, the Xerox Palo Alto Research Center, and the Cold Spring Harbor Lab. For more info, see: compbio.mit.edu and kellislab.com

Yi Zhang
Harvard University

Yi Zhang is an Investigator of the Howard Hughes Medical Institute and a Fred Rosen Chair Professor of the Department of Genetics and Department of Pediatrics of the Harvard Medical School. He is also a senior Investigator of the Program of Cellular & Molecular Medicine of the Boston Children's Hospital and Associate Member of the Harvard Stem Cell Institute and the Broad Institute. Before joining Harvard, he was a Kenan Distinguished Professor at UNC-Chapel Hill. His major interest has been the epigenetic basis of gene expression in early development, stem cell reprogramming, and reward-related learning and memory. He is also interested in how dysregulation of epigenetic enzymes contributes to various human diseases including cancer and drug addiction. Dr. Zhang is a highly accomplished protein biochemist and enzymologist known for his work in identifying and characterizing several classes of epigenetic enzymes that include the nucleosome remodeling and deacetylase (NuRD) complex, several histone methyltransferases (e.g. Ezh2/PRC2, Dot1L), the JmjC-containing histone demethylases, histone H2A ubiquitin E3 ligase PRC1, and the TET family of 5-methylcytosine dioxygenases. In recent years, he contributed to the understanding of epigenetics and chromatin remodeling in mammalian preimplantation development by uncovering a role of nucleosome assembly in nuclear pore complex formation of the male pronucleus, identifying the first transcription factor (NFYA) important for zygotic genome activation, and uncovering a DNA methylation-independent genomic imprinting mechanism that is critical for imprinted X-inactivation. He also identified and found ways to overcome the epigenetic barriers to somatic cell nuclear transfer reprogramming, which made the cloning of non-human primate possible. Zhang was named a Top 10 author of high impact papers in Genetics and Molecular Biology (2002-2006), and one of the most influential scientists in the world by ScienceWatch (2004-2014). He has published more than 180 high impact papers with more than 73,000 citations and an H-index of 113.
(https://scholar.google.com/citations?user=7UjSqHEAAAAJ&hl=en&oi=ao)

Invited Speakers

Swneke Bailey
McGill University

Dr. Swneke “Sven” Bailey is an assistant professor in the Departments of Surgery and Human Genetics at McGill University and a junior scientist at the research institute of the McGill University Health Centre. The overarching goal of his research program is to improve the survival of patients with cancers of the stomach, esophagus and lungs. His group is focused on understanding the role somatic mutations and alterations to the chromatin landscape in the progression of these cancers to metastasis. In addition, his group is developing bioinformatic approaches to interpret the function of non-coding genomic alterations to regulatory elements in cancer initiation and progression.

Wendy Bickmore
University of Edinburgh

Wendy Bickmore is Director of the MRC Human Genetics Unit, at the University of Edinburgh. Her undergraduate degree is in Biochemistry from the University of Oxford, and she then completed a PhD in molecular biology at the University of Edinburgh. Following a postdoc in human genetics, Wendy started her independent research group as a fellow of the Lister Institute of Preventive Medicine. She is fascinated by the three-dimensional organization of the human genome and how that influences genome function in health and disease. Her current research explores how the non-coding genome regulates gene expression. Wendy is a Fellow of; the Royal Society, the Royal Society of Edinburgh and of the Academy of Medical Sciences and is a member of the European Molecular Biology Organization. She is a governor of the Lister Institute of Preventive Medicine was awarded a CBE in the 2020 New Year’s Honours for service to women in science.

Marjorie Brand
Ottawa Hospital Research Institute

Marjorie Brand received her PhD in Molecular Biology from Universite Louis Pasteur in Strasbourg France. She is a Senior Scientist at the Sprott Center for Stem Cell Research in the Ottawa Hospital Research Institute and a Professor in the Department of Medicine at the University of Ottawa. She is also a member of the Royal Society of Canada's College of New Scholars, Artists and Scientists. Research in Dr. Brand’s lab is geared towards deciphering the mechanisms controlling cell fate decision in hematopoiesis and leukemia development.

Jacques Drouin
Institut de recherches cliniques de Montréal

Jacques Drouin is Director of the Laboratory of Molecular Genetics at the Institut de recherches cliniques de Montréal. He is Professor of Biochemistry at Université de Montréal and a member of its Molecular Biology Program. He is member of the Departments of Biochemistry, of Anatomy and Cell Biology, and of the Division of Experimental Medicine at McGill University. He is an elected member of the Academy of Sciences and Fellow of the Royal Society of Canada. He is also a member of the Canadian Academy of Health Sciences. He received a Honoris Causa Doctorate degree from Aix-Marseille Université. His contributions center on the molecular basis of pituitary gland function, development and diseases, and encompass discovery of transcriptional regulators that control cell differentiation, organogenesis and are implicated in diseases. These included discovery of 1) the Pitx subfamily of transcriptional regulators, 2) the Tbox factor Tpit, an essential regulator of pituitary cell fate that, when mutated, causes Isolated ACTH Deficiency, a fatal if untreated hormone deficiency and 3) the pioneer factor action of Pax7 to specify the intermediate pituitary fate and its mechanism of epigenetic action. Studies on transcriptional mechanisms of hormone action include repression by the glucocorticoid receptor (GR), extending from basic to genome-wide mechanisms and their implication for tumorigenesis and hormone resistance in Cushing’s disease. The latter studies include control of cell cycle in normal development and disease, as well as implementation of secretory capacity during cell differentiation. The group also investigated the role of Pitx genes in specification of hindlimb identity, in muscle development and in survival of dopaminergic neurons (Parkinson).

Vanessa Dumeaux
Concordia University

Dr. Dumeaux received her doctorate in Pharmacy in 2002 in France. She then obtained her Ph.D. in molecular epidemiology, a joint degree from the University of Tromso (Norway) and the University of Paris-South XI (France). During her PhD, she developed expertise in genomics in the group of Professeur Borresen-Dale at the Norwegian Radium Hospital which was among the pioneers in expression profiling of breast carcinomas in collaboration with groups at Stanford University. Dr Dumeaux co-initiated the post-genomic wing of the Norwegian Women and Cancer cohort to study the systems biology of breast cancer in a population-based context. She has had a long-standing interest in the development of RNA-based biomarker signatures in blood, which can be used to inform on exposure (exposome; eg. organic pollutants) and health status (eg. diagnostics of breast cancer, side-effects of radiotherapy). During her post-doc, she visited the Department of Applied Mathematics (University of Paris Descartes) and the Laboratory of Bioinformatics and Functional Genomics (Princeton University), and finally moved to Canada in 2009 to continue her research in bioinformatics and genomics at McGill University. Dr. Dumeaux is currently a Research Scientist at the PERFORM Center and Affiliate Assistant Professor in the Department of Biology at Concordia University, as well as a freelance consultant in data science and systems biology.

Edith Heard
EMBL Director General,
Professor of the Collège de France

Edith Heard is Director General of the EMBL. She studied Natural Sciences specialising in Genetics at Cambridge University and then obtained her PhD from the Imperial Cancer Research Fund in London. After a postdoc at the Institut Pasteur in Paris, she set up her group in 2001 at the Institut Curie where she became Director of the Genetics and Developmental Biology Unit in 2010. She was appointed as Professor of the Collège de France in 2012 to the Chair of Epigenetics and Cellular Memory, and in 2019 she became Director General of EMBL. Edith's laboratory studies epigenetics, focusing on the process of X-chromosome inactivation (XCI) whereby one of the two X chromosomes is silenced during female development. Her group has revealed the remarkable dynamics of epigenetic processes in development and disease. This has been recognised by many awards, most recently the L'Oréal-UNESCO For Women in Science International Award and her election to the National Academy of Sciences and the National Academy of Medicine.

Claudia Kleinman
McGill University

Dr Claudia Kleinman is an Assistant Professor in the Department of Human Genetics, Faculty of Medicine, at McGill University, Montreal, Canada. She is a fulltime Investigator at the Lady Davis Institute for Medical Research, an Associate Member of the McGill Centre for Translational Research in Cancer, and a researcher at the Ludmer Centre for Neuroinformatics & Mental Health. Her research exploits genome-wide technologies and data science to understand mechanisms of gene expression. She has an interdisciplinary training that combines molecular biology, computer science, statistics and evolutionary biology, which she applies to the study of pathological transcriptional and RNA processing events, focusing particularly on pediatric brain tumors. She has received awards from the Natural Sciences and Engineering Research Council of Canada (NSERC), Fond de Recherche du Québec – Santé (FRQS), Canadian Institutes of Health Research (CIHR) and currently holds a career award from the Fond de Recherche du Québec – Santé (FRQS). She is the recipient of the Bernard and Francine Dorval Prize, for her work mapping the development of the human brain at the single cell level to define the origins of pediatric brain tumors.

Marie Kmita
Institut de recherches
cliniques de Montréal

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

Ana Pombo
Max Delbrück Center for Molecular Medicine

Ana Pombo investigates mechanisms that regulate 3D genome folding and gene expression during mammalian development and in disease. After her doctorate work at the University of Oxford (UK), Ana was a recipient of a Royal Society Dorothy Hodgkin Fellowship. She started her independent group in 2000 at the MRC London Institute for Medical Sciences, associated with Imperial College London, before moving to the Max Delbrück Center for Molecular Medicine in Berlin, in Germany. Ana received the Robert Feulgen Prize in 2007, and was elected EMBO member in 2018, and she is currently the deputy scientific director of the Berlin Institute for Medical Systems Biology.

Michael Rudnicki
Ottawa Hospital Research Institute

Michael Rudnicki is a Senior Scientist and the Director of the Regenerative Medicine Program and the Sprott Centre for Stem Cell Research at the Ottawa Hospital Research Institute. He is Professor in the Department of Medicine at the University of Ottawa. Dr. Rudnicki is CEO and Scientific Director of the Canadian Stem Cell Network (SCN). Dr. Rudnicki's achievements have been recognized by numerous honours including being named a Tier 1 Canada Research Chair, an International Research Scholar of the Howard Hughes Medical Institute for two consecutive terms, a Fellow of the Royal Society of Canada, an Officer of the Order of Canada, and a Fellow of the Royal Society (London). He has been a founder in several spin-off biotechnology companies including Satellos Bioscience. Dr. Rudnicki is an internationally recognized thought leader in molecular genetics and regenerative medicine whose research has transformed our understanding of muscle development and regeneration and has fueled the development of novel stem cell-based approaches to treat muscular dystrophy. His work is consistently published in top journals including Cell, Nature, Nature Cell Biology, Nature Medicine, and Cell Stem Cell. He holds major research grants from CIHR, NIH, SCN, and several health charities. For the past 16 years, Dr. Rudnicki has led the Stem Cell Network (SCN), a transformative initiative involving over 175 investigators across Canada. As Scientific Director of the SCN, he has forged a national community that transformed stem cell research in Canada and brought research to the point where regenerative medicine is impacting clinical practice.

William Stanford
Ottawa Hospital Research Institute

Dr. William (Bill) L. Stanford, PhD earned his Bachelor's in Chemistry from Duke University, a doctorate in Immunology from UNC at Chapel Hill. Following a postdoctoral fellowship in functional genomics at the Lunenfeld, Dr. Stanford joined the University of Toronto in the Biomedical Engineering faculty in 2002 as a Tier 2 Canada Research Chair. Following a sabbatical in systems genetics at the Institute for Systems Biology with Lee Hood and David Galas, Dr. Stanford moved his lab in 2011 to the Ottawa Hospital Research Institute (OHRI). He is currently a Senior Scientist at the Sprott Centre for Stem Cell Research at the OHRI, a Full Professor at the University of Ottawa, Investigator in the Ottawa Institute of Systems Biology, Scientific Director of the Ottawa Human Pluripotent Stem Cell Facility, Scientific Director of the High Content Imaging and Screening Facility, and a Tier 1 Canada Research Chair in Integrative Stem Cell Biology.

Maria Elena Torres-Padilla
Helmholtz Zentrum München

Maria Elena Torres Padilla studied Biology at the National University of Mexico and obtained her PhD at the Pasteur Institute in Paris, France. She was a postdoctoral fellow at The Gurdon Institute, University of Cambridge, before starting her own lab at the IGBMC in Strasbourg, France. Currently she is Director of the Institute of Epigenetics and Stem Cells, Helmholtz Zentrum München, and Professor of the Chair of Stem Cell Biology, Faculty of Biology, LMU Munich. She is a member of the European Molecular Biolgoy Organisation (EMBO), of the Academia Europaea and a recipient of a number of awards for research in the field of epigenetics and cellular plasticity. She is also a member of the steering committee to the World Economic Forum Young Scientists community and contributed to the 2018 issuance of the WEF Code of Ethics for Researchers.

Iva Zovkic
University of Toronto
Mississauga

Iva Zovkic is a neuroscientist with a research focus on epigenetic mechanisms of memory formation across the lifespan. Her research focuses specifically on histone variants, which are a category of epigenetic regulators that have never been studied in the brain, leading to a Nature publication that identified H2A.Z as a novel memory suppressor and sparked a new area of epigenetic research in neuroscience. Her lab is especially interested in the unique feature of histone variants, particularly the observation that they are replication-independent (in contrast to replication-dependent canonical histones), which allows them to be synthesized in post-mitotic neurons, whereas canonical histones are not. Given that H2A.Z is a negative regulator of memory, she is now actively pursuing the hypothesis that H2A.Z accumulation over the life span is an active contributor to dementia and age-related cognitive decline. To this end, they published work demonstrating genome-wide accumulation of histone H2A.Z in aged mice and received a CIHR grant to study the role of H2A.Z in Alzheimer's disease. In addition, they recently published a paper demonstrating a new pharmacological strategy for reducing H2A.Z levels, with implications for translational studies of AD therapeutics. However, H2A.Z is just one of many histone variants, and the especially diverse group of H2A variants are excellent targets for memory regulation. They are currently interested in understanding how individual H2A variants contribute to memory and gene regulation, and the way that histone switching and chromatin reorganization supports memory formation and memory decline.

Workshop: Knowledge Translation

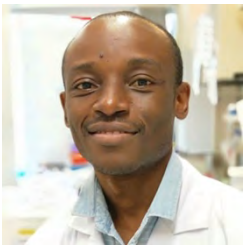
Knowledge Translation is defined by the Canadian Institutes of Health Research as a dynamic and iterative process that includes the synthesis, dissemination, exchange, and ethically sound application of knowledge to improve health, provide more effective health services and products, and strengthen the health care system. This definition has been adapted by other institutions, including the World Health Organization (WHO). Strategies for knowledge translation may vary according to the target audience (e.g., researchers, clinicians, educators, policymakers, the public) and the type of knowledge being translated. How can scientists, experts, trainees, and professionals in the field of epigenetics adopt KT strategies to disseminate and implement the findings of their research? How can we better facilitate the process of disseminating and implementing epigenetics knowledge for interdisciplinary research, clinical decision making, education, policy making and, in general, public understanding of science.

Kevin Sauvé
Canada's Michael Smith
Genome Sciences Centre

Kevin Sauvé is Head of Knowledge Translation at Canada's Michael Smith Genome Sciences Centre (GSC) at BC Cancer, where he manages a team in development and delivery of strategies and materials that help synthesize, exchange, and disseminate GSC's research. His expertise is in science communication consulting, writing and journalism. He has worked with the CBC and as a freelance science journalist, holds a Master of Journalism from UBC, concentrated on science, and a Bachelor's in Biology, from the University of Guelph, focused on neuroscience. He is also the recipient of a Canadian Institutes of Health Research, Health Research Communications Award.

Abstract Selected Speakers

Luis Abatti
Graduate Student
University of Toronto



George Anene-Nzelu
Assistant Professor
Montreal Heart Institute

Anjali Chawla
Graduate Student
McGill University

Geneviève Deblois
Assistant Professor
Universite de Montreal

Mehran Karimzadeh
Postdoctoral Fellow
Vector Institute

Benjamin Lebeau
Graduate Student
McGill University

Loïc Mangnier
Graduate Student
Universite de Laval

Shaghayegh Nouruzi
Graduate Student
University of British Columbia

Anna Prentice
Undergraduate Student
University of Toronto

Ahilya Sawh
Postdoctoral Fellow
University of Basel

Tabea Stephan
Graduate Student
University of British Columbia

CEEHRC Agenda

Wednesday, November 3rd, 2021

11:00 - 11:05	Opening Remarks - Martin Hirst
Session 1: Epigenetics in Development I Chairs: Sarah Kimmins (McGill University) and Elizabeth Elder (Université de Montréal)	
11:05 - 11:35	Maria Elena Torres-Padilla (Helmholtz Zentrum München) *Epigenetic mechanisms of early mammalian development*
11:35 - 12:05	Marie Kmita (Institut de recherches cliniques de Montréal) *Profiling of Polycomb Repressive Complexes reveals PRC2-independent PRC1 function in controlling limb developmental genes*
12:05 - 12:20	Anna Prentice (University of Toronto) *Locating and Dissecting Accessible, Conserved, Noncoding Elements of the Zebrafish Genome*
12:20 - 12:35	Mehran Karimzadeh (Vector Institute) *CiberATAC, A siamese residual convolutional neural network, deconvolves chromatin accessibility by learning from the transcriptome*
12:35 - 12:50	Breakout Sessions
Keynote Address Chair: Guillaume Bourque (McGill University)	
13:00 - 14:00	Manolis Kellis (Massachusetts Institute of Technology) *From genomics to therapeutics: Single-cell dissection and manipulation of disease circuitry*
14:00 - 14:15	Breakout Sessions
Poster Session 1	
14:30 - 16:00	See Poster Assignments (page 15)
Session 2: Epigenetics of Disease I Chair: Steve Bilodeau (Université Laval) and Daniel Sapozhnikov (McGill University)	
16:15 – 16:45	Marjorie Brand (Ottawa Health Research Institute) *Transcription factor-mediated leukemic transformation in T-ALL*
16:45 - 17:00	Geneviève Deblois (Université de Montréal) *Metabolic and epigenetic adaptations to microenvironment stress exposes vulnerabilities in breast cancer*
17:00 - 17:15	George Anene-Nzelu (Montreal Heart Institute) *Discovering Heart failure Genetic determinants though Epigenetic marks in Human Hearts*
17:15 - 17:30	Shaghayegh Nouruzi (University of British Columbia) *ASCL1 activates neuronal stem cell-like lineage programing through remodeling of the chromatin landscape in prostate cancer*
17:30 - 17:45	Breakout Sessions

Thursday, November 4th, 2021

Session 3: Regulation of the 3D Genome Chair: Marco Gallo (University of Calgary) and Michael Johnston (University of Calgary)	
11:00 – 11:30	Ana Pombo (Max Delbrück Center for Molecular Medicine) *Cell type specialization is encoded by specific 3D genome structures*
11:30 – 12:00	Wendy Bickmore (University of Edinburgh) *Enhancer function in the 3D genome*
12:00 – 12:15	Benjamin Lebeau (McGill University) *3D Chromatin Remodeling Potentiates Transcriptional Programs Driving Cell Invasion*
12:15 – 12:30	Loic Mangnier (Université Laval) *Cis-regulatory hubs constitute a powerful model to understand the impact of 3d organization in schizophrenia*
12:30 - 12:45	Breakout Sessions

Session 4: CEEHRC Trainee Keynote Chairs: Ariane Lismer (McGill University) and Sanne Janssen (University of British Columbia)	
13:00 - 14:00	Yi Zhang (Harvard University) *Distinct dynamics and functions of H2AK119ub1 and H3K27me3 in mouse preimplantation embryos*
14:00 - 14:15	Breakout Sessions

Poster Session 2	
14:30 - 16:00	See Poster Assignments (page 15)

Session 5: Epigenetics of Cell Renewal and Cell Fate Chairs: Jeffrey Dilworth (Ottawa Hospital Research Institute) and Edward Gardner (Ottawa Hospital Research Institute)	
16:15 - 16:45	Michael Rudnicki (Ottawa Hospital Research Institute) *Epigenetic Regulation of Muscle Stem Cell Self-Renewal*
16:45 - 17:15	Jacques Drouin (Institut de recherches cliniques de Montréal) *Mechanisms of pioneer factor action as master regulators of the epigenome and cell fate*
17:15 - 17:30	Tabea Stephan (University of British Columbia) *Chromatin dynamics and transcription factor networks driving hepatic fate in iPSC derived hepatocytes*
17:30 - 17:45	Breakout Sessions

Friday, November 5th, 2021

Session 6: Epigenetics in Development II Chair: Serge McGraw (Université de Montréal) and Hossein Davarinejad (University of Ottawa)	
11:00 - 11:30	Edith Heard (EMBL) *Epigenetic Dynamics in Development and Disease: The X-inactivation Paradigm*
11:30 - 12:00	Vanessa Dumeaux (Concordia University) *Challenges and opportunities in profiling the sperm epigenome*
12:00 – 12:15	Ahilya Sawh (University of Basel) *Conformational acrobatics of entire chromosomes during embryonic development*
12:15 - 12:30	Breakout Sessions

Workshop: Epigenetics Knowledge Translation Chair: Jim Davie	
12:45 - 13:45	Kevin Sauve (Genome Sciences Centre)

Session 7: Neuroepigenetics Chair: Nathalie Berube (Western University) and Lisa-Marie Legault (Université de Montréal))	
14:00 - 14:30	Iva Zovkic (University of Toronto Mississauga) *An emerging role for histone variants in learning and memory*
14:30 - 15:00	Claudia Kleinman (McGill University) *Epigenome engraving of cellular lineage in childhood brain tumors*
15:00 – 15:15	Anjali Chawla (McGill University) *Cell-type specific open chromatin signatures in the brains of individuals diagnosed with major depressive disorder and died by suicide*
15:15 - 15:30	Breakout Sessions

Session 8: Epigenetics in Cancer Chair: Nada Jabado (McGill University) and Alvin Qiu (University of British Columbia)	
15:45 - 16:15	William Stanford (Ottawa Health Research Institute) *Derepression of alternative DNA repair networks drives refractory acute myeloid leukemia*
16:15 - 16:45	Swneke Bailey (McGill University) *A predominant enhancer co-amplified with SOX2 is necessary for its expression and uncovers potential therapeutic strategies for squamous cancers*
16:45 – 17:00	Luis E. Abatti (University of Toronto) *Epigenetic misactivation of a distal developmental enhancer cluster drives SOX2 overexpression in multiple cancer subtypes*
17:00 - 17:15	Breakout Sessions
17:15 – 17:30	Closing Remarks – Martin Hirst
17:30 – 18:00	CEEHRC Trainee Discussion

Poster Assignments

Wednesday, November 3	Thursday, November 4
Abatti, Luis	Ahmed, Abdalla
Akbari, Vahid	Azarafshar, Pariya
Awamleh, Zain	Berthold, Anne
Ayala Esparza, Jose Mauricio	Budzynski, Marek
Azevedo Portilho, Nathalia	Chen, Carol
Bari, Khaleda	Chen, Raymond
Barr, Emily	Chen, Jocelyn
Barutcu, A. Rasim	Cinkornpumin, Jessica
Bilenky, Misha	Dasgupta, Anirban
Braeutigam, Katharina	Desai, Kinjal
Brakstad, Jordan	Distéfano-Gagné, Félix
Cardin, Valerie	Duran Bishop, Gilberto
Carles, Annaïck	Elder, Elizabeth
Chang, Kai-Wei	Fava, Vinicius
Clément, Andrée-Anne	Frosi, Gabriella
Davarinejad, Hossein	Fukano, Marina
Desaulniers, Daniel	Glass, Karen
Doiron, Karine	Greenwood, Celia
Edgar, Rachel	Ha, Amanda
Emam, Mehdi	Hauduc, Axel
Filice, Mario	He, Housheng
Fuglerud, Bettina	Hoekstra, Matthew
Gardner, Edward	Hyacinthe, Jeffrey
Goodman, Sarah	Janna, Ashley
Groza, Cristian	Keshavarzian, Tina
Han, Jiyoung	Kwan, James
Hossain, Ishtiaque	Labbé, David
Inkster, Amy	Légaré, Cécilia
Joshi, Monika	Lemieux, Anthony
Kopp, Audrey	Lubachowski, Mathew
Kwon, Sin Young	Malone, Kiera
Langford-Avelar, Alexandra	Mangnier, Loic
Legault, Lisa-Marie	McLean, Timothy
Li, Shuxiang	Mercier, Gabrielle
Maitra, Malosree	Nad, Ivana
Mancini, Mathieu	Nguyen, Thomas
Manu, Diana	Nouruzi, Shaghayegh
Meleady, Laura	Onabote, Oladapo
Mitsuhashi, Haruka	Paul, Racheal
Neault, Mathieu	Phillips, Margaret
Nikolic, Ana	Qiu, Alvin
Nuyens, Alexandra	Reed, Madison
Pena-Ortiz, Miguel	Schulz, Mathieu
Priam, Pierre	Shafiq, Sarfraz
Rafique, Tanzeem	Simon, Marie-Michelle
Rowland, Megan	Song, Mengyi
Saini, Deepak	Stefanelli, Gilda
Segal, Dana	Tam, Derek
Siklenka, Keith	Thibeault, Kathrine
Sokolowski, Dustin	Uuskula-Reimand, Liis
Srour, Nivine	Vasileva, Denitsa
Tang, Helen	Wang, Fangwu
Tremblay, Roch	Welton, Jessica-Lynne
Weaver, Ian	Zeng, Yixiao
Yang, Kimmy	Zimmer, Samuel
Zhuang, Qinwei	

Abstract Selected Talks

EPIGENETIC MISACTIVATION OF A DISTAL DEVELOPMENTAL ENHANCER CLUSTER DRIVES SOX2 OVEREXPRESSION IN MULTIPLE CANCER SUBTYPES

Luis E. Abatti[1], Linh Huynh[2], Michael M. Hoffman[2], Jennifer A. Mitchell[1]
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SRY-box transcription factor 2 (SOX2) is a key transcription factor in embryonic and reprogrammed stem cells that cooperates with a wide network of transcription factors to regulate pluripotency-associated genes. SOX2 further plays a crucial role in the development and homeostasis of many tissues, including the trachea, esophagus, lungs, and stomach. Within these tissues, *SOX2* remains highly expressed in adult progenitor basal cells, which continuously give rise to mature epithelial cells. *SOX2* overexpression contributes to tumorigenesis in more than 25 different cancer types, including breast cancer and lung cancer. In both these cases, overexpression of *SOX2* is associated with increased replication, aggressive tumour grades, and poor patient outcomes due to participation in the formation and maintenance of tumour-initiation progenitor cells. We identified two novel genomic regions, SRR124 and SRR134, with increased enhancer-associated epigenetic features in *SOX2*-overexpressing MCF-7 breast adenocarcinoma cells. These features include open chromatin, histone modifications H3K4me1 and H3K27ac, and the binding of over 40 transcription factors. Both SRR124 and SRR134 regions are absent of enhancer features in normal mammary epithelium, indicating that they become active as epithelial cells dedifferentiate to a cancerous state. ATAC-seq revealed that the SRR124 and SRR134 regions are open in both breast cancer and lung cancer patients, and that open chromatin at these regions is positively and significantly correlated to open chromatin at the *SOX2* promoter. RNA-seq indicated that cancer patients with the most chromatin accessibility at these two regions also significantly overexpress *SOX2.* Furthermore, reporter assays demonstrated that both the SRR124 and SRR134 regions act as enhancers in breast cancer and lung cancer cells. CRISPR/Cas9-induced homozygous deletion of the SRR124–134 region significantly reduced *SOX2* expression in both breast cancer MCF-7 cells and lung cancer PC-9 cells. *SOX2* downregulation further affected chromatin accessibility and resulted in the downregulation of multiple genes associated with epithelium development in MCF-7 cells, including *AREG*, *BTC*, *EREG*, and *SHH*. Finally, cross-species analysis showed that both SRR124 and SRR134 sequences have high conservation across mammals and that these regions display enhancer features during development of the digestive and respiratory systems in both human and mouse species. Based on these findings, we propose that the SRR124 and SRR134 regions normally control *SOX2* expression during foregut development and that aberrant accessibility of these regions drives *SOX2* overexpression in breast and lung tumours. Understanding the mechanisms by which these novel regulatory regions facilitate *SOX2* overexpression will help identify transcriptional network changes associated with tumour-initiating progenitor cells.

DISCOVERING HEART FAILURE GENETIC DETERMINANTS THOUGH EPIGENETIC MARKS IN HUMAN HEARTS

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Identifying genetic markers for complex heterogeneous diseases such as heart failure is challenging, and often requires large cohort sizes in genome-wide association studies (GWAS) in order to meet the stringent threshold of genome-wide statistical significance. Conversely, chromatin quantitative trait loci (QTL), elucidated through direct epigenetic profiling of specific human tissues, may contribute towards prioritising novel variants for disease-association. Here, by performing epigenetic profiling for enhancer H3K27ac ChIP-seq in 70 human hearts, and applying the G-SCI test, we captured 1680 non-coding genetic variants that alter acetylation peak height, designated as histone acetylation QTLs (haQTLs). RNA-seq performed on the same heart samples proved a subset of haQTLs to have significant association to gene expression (expression QTLs), either in *cis*, or through long range interactions, identified by Hi-C and HiChIP performed on a subset of hearts. Furthermore, a concordant relationship between the gain or disruption of transcription factor (TF) binding motifs, inferred from alternative alleles at the haQTLs, implied an association between these specific TF and local histone acetylation in human hearts. Finally, 62 unique loci were identified by colocalisation of haQTLs with the sub-threshold loci of heart-related GWAS datasets. These loci may indeed mediate their effect through modification of enhancer H3K27-acetylation enrichment and their corresponding gene expression differences.

METABOLIC AND EPIGENETIC ADAPTATIONS TO MICROENVIRONMENT STRESS EXPOSES VULNERABILITIES IN BREAST CANCER

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Tumor progression and therapeutic resistance are characterized by changes in cell identity, which is regulated in part by reprogramming of the epigenetic landscapes of cancer cells. Cancer progression is also supported by the rewiring of

cellular metabolism, which enables cancer cells to fulfill elevated energetic, biomass and antioxidant requirements. Cancer cells must balance energy-producing and -consuming metabolic processes to fuel tumor growth, while adapting to the dynamic changes in nutrient and oxygen availability through a process called metabolic flexibility. Changes in metabolites availability can also contribute to the remodelling of chromatin landscapes, providing a way for cancer cells to adjust transcriptional programs in response to their metabolic states. In turn, the reprogramming of epigenetic landscapes can modulate transcriptional programs that support metabolic flexibility to allow cancer cell adaptations in response to microenvironmental and cytotoxic stresses. Using the poor prognosis Triple-Negative Breast Cancer (TNBC), we have shown that cytotoxic stress and perturbations of metabolic states can induce dynamic changes in chromatin landscapes and associated complexes. We further show that epigenetic reprogramming, including changes in EZH2-mediated epigenetic programs, can support TNBC metabolic adaptations by regulating transitions between the metabolic states of TNBC cells. While these reciprocal metabolic and epigenetic changes contribute to cell survival under stress, they also reveal vulnerabilities that could be targeted in TNBC.

CIBERATAC, A SIAMESE RESIDUAL CONVOLUTIONAL NEURAL NETWORK, DECONVOLVES CHROMATIN ACCESSIBILITY BY LEARNING FROM THE TRANSCRIPTOME

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Background. Widely used single-cell transcriptomic assays identify dysregulated genes but fail to identify the cis-regulatory elements regulating those genes. Even with paired single-cell multi-omics, identifying the most important cis-regulatory elements in a cell type of interest remains challenging. Resolving these limitations allows for a better understanding of gene regulation in both modalities at the single-cell level.

Method. Our method, Cis-regulatory element Identification By Expressed RNA and ATAC-seq, CiberATAC, identifies active cell-type–specific cis-regulatory elements in scRNA-seq data and ATAC-seq data (bulk or single-cell). Unlike other deconvolution methods, CiberATAC does not require access to a deconvolution reference, making it applicable to rare and new cell types. CiberATAC introduces a novel deep learning framework that learns semantic feature representations to model epigenomic and transcriptomic data. It adopts a novel contrastive learning algorithm by using a siamese residual convolutional neural network to model bulk or single-cell chromatin accessibility as well as cell-type–specific transcription within 20 kbp (±10 kbp) of each cis-regulatory element to predict its cell-type–specific activity. In addition to local transcriptome and epigenome input, CiberATAC also requires global transcriptome embeddings of single-cells. We developed a novel variational auto-encodoer (VAE) inspired by biological connections, as opposed to fully connected layers, to represent global transcriptome identities as input for the CiberATAC model. We inferred the biological connections according to the genes each transcription factor regulates (as annotated by the molecular signature database).

Results. CiberATAC's VAE achieved adjusted Rand index (ARI) of 0.8 on primary blood mononuclear cells (PBMC) dataset while the state-of-the-art algorithm for this task, scVI, only achieved ARI of 0.6. CiberATAC identified the active enhancers with accuracy of 0.75 (balanced dataset) on held-out cell types of PBMC dataset as well as the held-out 10X multi-omic Lymphoma dataset. Using *in-silico* perturbation of the gene promoter transcription levels within a trained network allows for identifying cell-type–specific enhancers. Doing so we identified reported monocyte-specific enhancers with 0.87 precision (predicted enhancers / H3K27ac peaks). To our best knowledge, CiberATAC is the only algorithm deconvolving the chromatin in transcriptionally similar cell types while characterizing cell-type–specific enhancers.

3D CHROMATIN REMODELING POTENTIATES TRANSCRIPTIONAL PROGRAMS DRIVING CELL INVASION

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It is clear that epigenetic mechanisms often play an important role in driving tumor progression towards metastasis. But it remains unknown whether a global deregulation of chromatin architecture, including topologically associated domains (TADS), might contribute to cancer progression. CTCF is a key regulator of 3D chromatin structure that undergoes copy number loss in over half of all breast cancers, but the impact of this defect on tumor progression, epigenetic programming and chromatin architecture remains obscure. Using CTCF hemizygous models, including lines derived from PDX models of triple-negative breast cancer, we find that reduced pools of CTCF potentiates cell invasion. Underlying this phenotype is the activation of oncogenic networks including the PI3K signaling cascade and upregulation of the classical oncogene Snai1. Integrating RNA-seq, ChIP-seq and Hi-C data revealed that such pathways are deregulated largely due to the loss of CTCF insulation, allowing aberrant reprogramming of H3K27 acetylation at enhancer/promoter regions across the genome and a reorganization of long-range chromatin contacts at the subTAD level. Using this information, we discovered that cell invasion of CTCF hemizygous cells proves to be acutely sensitive to mTOR inhibitors. Thus, reduced pools of CTCF leads to reprogramming of chromatin architecture facilitating pro-invasive transcriptional programs, but understanding these mechanisms reveals therapeutic vulnerabilities.

LOCATING AND DISSECTING ACCESSIBLE, CONSERVED, NONCODING ELEMENTS OF THE ZEBRAFISH GENOME

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Conserved, noncoding elements are genomic elements maintained over significant evolutionary time. Many of these elements have been found to represent enhancers governing various developmental processes. When the additional criterion of chromatin accessibility is applied, a subset of these CNEs: accessible, conserved, noncoding elements—aCNEs-−—can be identified.

This project seeks to identify putative cardiac enhancers among aCNEs accessible in mid-gastrulation zebrafish embryos. To achieve this goal, aCNEs will be examined through the lens of the gata5/6 transcription factors. These factors are master regulators of heart development. Conserved in humans as *GATA4/6*, the ablation of these genes in zebrafish results in a completely heartless phenotype. Furthermore, as pioneer factors expressed earlier than most canonical heart markers (e.g., *nkx2.5*), gata5/6 may exert an impact on chromatin accessibility that allows the epigenetic landscape of early cardiac development to be further elucidated.

These putative cardiac enhancer aCNEs were selected based on their accessibility in *gata5*-expressing cells and their sensitivity to *gata5/6* knockdown in zebrafish. Additionally, elements were prioritized if their orthologous human regions were identified as GATA4 binding sites in cardiomyocytes (CMs) or accessible regions in cardiac progenitor cells (CPCs). The selected elements were then assessed for enhancer activity in zebrafish.

In total, 4,925 aCNEs were identified in the zebrafish genome (corresponding to 8,656 human regions). Of these, 302 exhibited enriched accessibility in *gata5*-expressing cells, and 65 lost their open chromatin signature upon *gata5/6* knockdown. 47 aCNEs displayed both these features, three of which were also GATA4 binding sites in human CMs *and* accessible in human CPCs. One of these regions—8h_aCNE1—is intronic within *runx1t1,* a synteny conserved in the human aCNE. *runx1t1* is a transcriptional co-repressor of *RUNX1*, and a fusion of these two genes confers a phenotype that includes congenital heart disease (CHD). 8h_aCNE1 itself also contains a hit for an ultra-rare variant in a patient displaying a CHD phenotype. For these reasons, 8h_aCNE1 was selected for subsequent testing in a reporter assay. This aCNE demonstrated strong cardiac enhancer activity in several F_1 candidates at 48 hours post-fertilization.

These results suggest that aCNEs represent a group of evolutionarily conserved enhancers that, when subset using selected datasets, may offer a strong enrichment of tissue-specific expression and function.

CONFORMATIONAL ACROBATICS OF ENTIRE CHROMOSOMES DURING EMBRYONIC DEVELOPMENT

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Eukaryotic genome organization is ordered and multi-layered, from the nucleosome to chromosomal scales. The spatial organization of chromatin impacts essential processes including DNA replication, recombination, and gene expression. Current models suggest that epigenetically similar chromatin domains assemble into either active or inactive compartments, but the physical configurations of chromosomes *in vivo* and their cell-cell variability are poorly understood. We developed high-throughput, multiplexed, sequential DNA fluorescence *in situ* hybridization (FISH) to directly visualize and trace the contours of entire chromosomes ('*chromosome tracing*') in intact *C. elegans* embryos. Chromosome tracing enables the localization of 10s-1000s of fluorescently labeled genomic targets with sequence specificity along individual chromosomes in each sample, leading to a wealth of positional data that we use to map chromosome architecture at both the population and single-chromosome level. We found that early embryonic chromosomes organize into barbell-like configurations, where epigenetically similar domains are unexpectedly segregated. The barbell is subsequently remodeled during gastrulation to consolidate inactive genomic regions in space over large genomic distances. Using unsupervised single-chromosome clustering to examine cell-to-cell variability, we uncovered sub-populations of conformations defined by differing nuclear lamina association, biophysical folding properties, and the strength and positioning of compartment boundaries. Disruption of lamina association caused systemic shrinking of entire chromosomes and increased interactions between epigenetically similar domains. These findings reveal that chromosomes exhibit diverse spatial organization during embryogenesis and identify a novel topological role for the lamina in large-scale chromosome stretching.

CHROMATIN DYNAMICS AND TRANSCRIPTION FACTOR NETWORKS DRIVING HEPATIC FATE IN IPSC DERIVED HEPATOCYTES

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How the hepatocyte transcriptome is established during development, including how the underlying chromatin dynamics control cell identity and function is still unclear. While several transcription factors (TFs) responsible for aspects of liver development have been identified in mouse models, chromatin dynamics and regulating TFs in human cells are not well studied. The lack of consistency and functionality of iPSC derived mature hepatocytes suggest a knowledge gap. Here, we describe the changing epigenome during in vitro hepatocyte differentiation and identify missing links in the transcription factor network. We hypothesize that genome-wide mapping of enhancers during hepatic differentiation can identify TFs governing a hepatic chromatin landscape. By disrupting these TFs using knockdown studies, we will dissect their role in the transcription factor network that controls hepatic identity. We are using *in vitro* differentiation of iPSCs to hepatic progenitors as a model of liver cell differentiation. By mapping post-translational histone tail modifications across the genome at several timepoints we developed a comprehensive map of enhancers and promoters and their activation status during hepatic cell differentiation. We are then using TF motif enrichment analysis to identify factors directing these dynamics. We further developed an inducible knockdown system to remove TFs of interest during hepatic differentiation. Currently, we are investigating the role of TBX3 on the specification of the hepatic chromatin. We found a highly dynamic epigenome with 99% of all enhancers changing their activation status throughout the differentiation. While some enhancers cycle between active and repressed without a clear association with a differentiation stage, others are activated or repressed at a specific stage. We identified a small group of enhancers (n =3600) which are primed for activation in definitive endoderm and which are not consecutively shutdown, as the majority of enhancers are, but activated during further differentiation towards hepatocytes. This small group of driver enhancers appears crucial for hepatic cell fate as it includes enhancers controlling the promoters of HNF4a and CEBPa which are master regulators of hepatocyte cell fate. Our analysis suggests TBX3 as a potential regulator of these primed to activated enhancers. Effectively specifying the hepatic lineage from other endodermal lineages through precise enhancer activation would add a new role to the importance of TBX3 during hepatic development. A better understanding of human liver development and the underlying changes in the chromatin regulation will aid in the development of efficient methods to generate *in vitro* derived mature hepatocytes to potentially treat end-stage liver diseases. This study will also be of value for research into the regulation of chromatin during liver regeneration and oncogenesis.

Abstracts

EPIGENETIC MISACTIVATION OF A DISTAL DEVELOPMENTAL ENHANCER CLUSTER DRIVES SOX2 OVEREXPRESSION IN MULTIPLE CANCER SUBTYPES

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SRY-box transcription factor 2 (SOX2) is a key transcription factor in embryonic and reprogrammed stem cells that cooperates with a wide network of transcription factors to regulate pluripotency-associated genes. SOX2 further plays a crucial role in the development and homeostasis of many tissues, including the trachea, esophagus, lungs, and stomach. Within these tissues, *SOX2* remains highly expressed in adult progenitor basal cells, which continuously give rise to mature epithelial cells. *SOX2* overexpression contributes to tumorigenesis in more than 25 different cancer types, including breast cancer and lung cancer. In both these cases, overexpression of *SOX2* is associated with increased replication, aggressive tumour grades, and poor patient outcomes due to participation in the formation and maintenance of tumour-initiation progenitor cells. We identified two novel genomic regions, SRR124 and SRR134, with increased enhancer-associated epigenetic features in *SOX2*-overexpressing MCF-7 breast adenocarcinoma cells. These features include open chromatin, histone modifications H3K4me1 and H3K27ac, and the binding of over 40 transcription factors. Both SRR124 and SRR134 regions are absent of enhancer features in normal mammary epithelium, indicating that they become active as epithelial cells dedifferentiate to a cancerous state. ATAC-seq revealed that the SRR124 and SRR134 regions are open in both breast cancer and lung cancer patients, and that open chromatin at these regions is positively and significantly correlated to open chromatin at the *SOX2* promoter. RNA-seq indicated that cancer patients with the most chromatin accessibility at these two regions also significantly overexpress *SOX2.* Furthermore, reporter assays demonstrated that both the SRR124 and SRR134 regions act as enhancers in breast cancer and lung cancer cells. CRISPR/Cas9-induced homozygous deletion of the SRR124–134 region significantly reduced *SOX2* expression in both breast cancer MCF-7 cells and lung cancer PC-9 cells. *SOX2* downregulation further affected chromatin accessibility and resulted in the downregulation of multiple genes associated with epithelium development in MCF-7 cells, including *AREG*, *BTC*, *EREG*, and *SHH*. Finally, cross-species analysis showed that both SRR124 and SRR134 sequences have high conservation across mammals and that these regions display enhancer features during development of the digestive and respiratory systems in both human and mouse species. Based on these findings, we propose that the SRR124 and SRR134 regions normally control *SOX2* expression during foregut development and that aberrant accessibility of these regions drives *SOX2* overexpression in breast and lung tumours. Understanding the mechanisms by which these novel regulatory regions facilitate *SOX2* overexpression will help identify transcriptional network changes associated with tumour-initiating progenitor cells.

LYSINE DEMETHYLASE 8 EPIGENETICALLY REGULATES CARDIAC METABOLISM TO PREVENT MYOCARDIAL DETERIORATION IN HEART FAILURE

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Dilated cardiomyopathy (DCM) is the leading cause of heart failure. Deposition of histone modifications is deregulated in end-stage DCM, but how this affects the initiation and progression of disease is unknown. Here we show that lysine demethylase 8 (Kdm8), which demethylates the di-methylated form of lysine 36 of H3 (H3K36me2), regulates cardiac metabolism and maintains homeostasis to protect against DCM. Deletion of *Kdm8* in mouse cardiomyocytes causes DCM which progresses to fulminant heart failure. Transcriptional profiling on *Kdm8* mutant hearts revealed downregulation of gene networks controlling mitochondrial metabolism before onset of cardiac dysfunction. Consistently, mitochondria structure was altered, and its function decreased prior to disease onset. These changes were associated with deregulation of the cardiac metabolome, with a significant reduction in the metabolites required for NAD^+ synthesis. We show that loss of Kdm8 activates the T-Box Transcription Factor 15 (Tbx15), a known metabolic repressor. Luciferase assays demonstrate that Tbx15 directly inhibits the promoter of nicotinamide phosphoribosyltransferase (*Nampt*), an enzyme which converts nicotinamide to the NAD^+ precursor, nicotinamide mononucleotide. Boosting mitochondrial function by NAD^+ administration restored cardiac function in *Kdm8* mutants. Importantly, Kdm8 target genes and pathways which control mitochondrial metabolism are also dysregulated in human hearts affected by DCM. Thus, Kdm8 maintains cardiac metabolic homeostasis, and disrupting its function initiates cardiac deterioration leading to heart failure. Furthermore, our findings support NAD^+ administration as a potential strategy for preventing heart failure.

DETECTION OF IMPRINTED DNA METHYLATION USING NANOPORE LONG-READ SEQUENCING

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Imprinting is a critical part of normal embryonic development in mammals, controlled by defined parent-of-origin (PofO) differentially methylated regions (DMRs) known as imprinting control regions (ICRs). As we and others have shown, direct nanopore sequencing of DNA provides a mean to detect allelic methylation and to overcome the drawbacks of methylation array and short-read technologies. Here we leverage publicly available nanopore sequence data for 12 standard B-lymphocyte cell lines to present the first genome-wide mapping of imprinted intervals in humans using this technology. We were able to phase 95% of the human methylome and detect 94% of the well-characterized imprinted DMRs. In addition, we found 28 novel imprinted DMRs (12 germline and 16 somatic), which we confirmed using whole-genome bisulfite sequencing (WGBS) data. Analysis of WGBS data in mus musculus, rhesus macaque, and chimpanzee suggested that 12 of these are conserved. We also detected subtle parental methylation bias blocks spanning multiple kilobases with several features in common at seven known imprinted clusters. These results expand the current state of knowledge of imprinting, with potential applications in the clinic. We have also demonstrated that nanopore long reads, can reveal imprinting using only parent-offspring trios, as opposed to the large multi-generational pedigrees that have previously been required.

DNA METHYLATION SIGNATURE ASSOCIATED WITH PATHOGENIC VARIANTS IN ASXL1 AND BOHRING-OPITZ SYNDROME: A NEW TOOL FOR FUNCTIONAL CLASSIFICATION OF VARIANTS IN ASXL GENES

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The additional sex combs-like (ASXL) gene family—encoded by *ASXL1*, *ASXL2*, and *ASXL3*—is crucial for mammalian development through transcriptional regulation of the *HOX* gene cluster. Pathogenic variants in *ASXL* genes are associated with three phenotypically distinct neurodevelopmental syndromes. Our previous work has shown that syndromic conditions caused by pathogenic variants in epigenetic regulatory genes show consistent patterns of DNA methylation (DNAm) in peripheral blood: DNA methylation signatures. With evidence of the role of ASXL1/2 in chromatin modification, particularly deubiquitylation of lysine (K119) on histone 2A, we hypothesized that pathogenic *ASXL1* variants underlying Bohring-Opitz syndrome (BOS) have a unique DNAm signature. We profiled whole-blood DNAm for 17 *ASXL1* variants, 1 *ASXL2* variant, 3 *ASXL3* variants, and 35 sex- and age-matched typically developing individuals, using Illumina's Infinium EPIC array. Using linear regression modelling, we identified 763 differentially methylated CpG sites (q <0.05, delta beta > 10%) in individuals with BOS (n=8). Differentially methylated sites overlap 323 unique genes, including *HOXA5* and *HOXB4*, supporting the functional relevance of DNAm signatures. A machine-learning classification model based on the DNAm signature classified a validation cohort of BOS individuals (n=6) with *ASXL1* variants and controls (n=100) correctly, demonstrating high sensitivity and specificity of the model. We used the model to classify variants of uncertain significance in *ASXL1* (n=3) as BOS-like or control-like; each classification was congruent with the patients' clinical phenotypes. We also used the machine-learning classification model to classify variants in *ASXL2* and *ASXL3*. The variant in *ASXL2* classified as BOS-like, whereas variants in *ASXL3* classified as control-like. This suggests that *ASXL2* has a DNAm profile overlapping the *ASXL1* DNAm profile, whereas *ASXL3* likely has a unique profile. Our DNA methylation data provide unique insights into *ASXL* syndrome pathophysiology. The DNAm signature we generated is gene- and syndrome-specific, it classifies all individuals with Sotos, Weaver, and Kabuki syndromes as benign, accurately differentiating pathogenic *ASXL1* variants causing BOS from variants underlying other chromatin modifying neurodevelopmental disorders.

THE DYNAMICS OF EARLY AND LATE CHROMATIN REMODELING DURING IFNγ MACROPHAGE ACTIVATION

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Macrophages are highly conserved phagocytes of the innate immune system, present in nearly all tissues of adult mammals, crucial for processes ranging from tissue homeostasis to host defense. The high diversity potential in macrophage activity has often been attributed to their vast collection of receptors, coupled to complex signaling pathways. Changes to the epigenetic landscape of macrophages mediated by transcription factors have now arisen as key modulators of the macrophage response to stimuli. Interferon-γ (IFNγ) is a crucial cytokine for macrophage activation, responsible for beefing up its phagocytic ability and inflammatory potential. Most studies characterizing the epigenetic changes following IFNγ stimulation in macrophages, have only focused on its priming effect in relationship to other stimulants or have used limited time points. Consequently, there is a lack of knowledge on how chromatin accessibility evolves globally throughout and beyond early IFNγ activation, the regulatory elements involved, and which transcription factors may be acting during this process. The aim of the present project is to characterize the chromatin accessibility changes in bone marrow-derived macrophages (BMDMs) following IFNγ stimulation and to identify the molecular actors in their epigenetic reprogramming. We have

used the assay for transposase-accessible chromatin sequencing (ATAC-seq) at 9 different time points (0h to 48h), as well as use both chromatin immunoprecipitation sequencing (ChIP-seq) and ATAC-seq footprinting to identify the key transcription factors involved. Lastly, we use ChIP-seq data from histone marks to identify regulatory elements within the analysis. The results will expand our knowledge on how macrophages respond to activation to IFNγ, specifically the waves of regulatory elements being opened and closed, their related transcriptional programs and the spatial / temporal involvement of transcription factors at a global level.

CHARACTERIZING THE ROLE OF HISTONE H3.3 K36 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 on the H3.3 encoding genes (*H3F3A* and *H3F3B*) which result in amino acid substitutions on H3.3 tail, are responsible for different types of malignancies. However, germline mutations on H3.3 correlate with neurodevelopmental disorders in human patients. Recently, a male patient suffering from developmental delays, seizure, and craniofacial abnormalities with the *H3f3A* K36E germline point mutation has been reported. H3.3K36me3 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, K36E substitution may disrupt the activity of SETD2 *in-cis*, and potentially disrupt ZMYND11 binding to H3.3K36. For this project, we generated Direct-Knock-In (DKI) *H3f3a* K36E mouse model to study this mutation, and by investigating the effects of the mutation on mice behavior, we have observed, hyperactivity, anxiety, self-mutilation and in general ASD-like behaviors. Next, we will be looking at various brain populations, as well as the consequences on other tissues and organs. Furthermore, by using transcriptomic and epigenomic tools and Next-Generation-Sequencing (NGS) we will analyze the effects of this mutation on gene expression pattern and post-translational modifications, respectively. This study will give further insights into the role of K36 residue and K36E substitution in chromatin remodeling and brain neurodevelopment.

THE DNMT1 INHIBITOR GSK-3484862 MEDIATES GLOBAL DEMETHYLATION IN MURINE EMBRYONIC STEM CELLS

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DNA methylation plays an important role in regulating gene expression in mammals. The covalent DNMT1 inhibitors 5-azacytidine and decitabine are widely 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. Here, we sought to determine whether GSK-3484862 can induce demethylation more effectively than 5-azanucleosides. Murine embryonic stem cells (mESC) 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. In this study, 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 M 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 6 days of treatment with GSK-3484862, similar to the methylation level observed in Dnmt1 deficient mESCs. Collectively, GSK-3484862 mediates striking demethylation in mESCs with minimal non-specific toxicity.

TRA1 REGULATES CHRONOLOGICAL AGING IN YEAST

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Gene expression undergoes considerable changes during the aging process. The mechanisms regulating the transcriptional response to cellular aging remain poorly understood. Here, we employ the budding yeast *Saccharomyces cerevisiae* to better understand how organisms adapt their transcriptome to promote longevity. In yeast, chronological lifespan (CLS) assays are used to study the survival of non-dividing cells at stationary phase over time, providing insights into the aging process of post-mitotic cells in other organisms. Tra1 is an essential component of both the yeast SAGA/SLIK and NuA4 complexes, where it recruits these complexes to acetylate histones at targeted promoters. Importantly, Tra1 regulates the transcriptional response to multiple stresses. To evaluate the role of Tra1 in chronological aging, we took advantage of a previously characterized mutant allele that carries mutations in the *TRA1* PI3K domain ($tra1_{Q3}$) (Berg *et al.*, G3, 2018). We find that loss of functions associated with ($tra1_{Q3}$) shortens lifespan.

Transcriptional profiling reveals that genes differentially regulated by Tra1 during the aging process are enriched for components of the response to proteotoxic stress. Indeed, small heat shock proteins (*HSP26, HSP42, HSP104*) are significantly downregulated in chronologically aged $tra1_{Q3}$. *HSP26, HSP42* and *HSP104* are essential for CLS with a mutant lacking all 3 genes displaying reduced longevity. In addition, expression of catalases (*CTA1*, *CTT1*) involved in hydrogen peroxide detoxification decreases in chronologically aged $tra1_{Q3}$ cells. Consequently, they also display increased levels of toxic reactive oxygen species (ROS). Thus, Tra1 emerges as an important regulator of longevity in yeast via multiple mechanisms. Supported by CIHR.

THE BIOLOGICAL EMBEDDING OF CHILDHOOD ADVERSITY THROUGH THE EPIGENOME

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Youth who experience early adversity or stress early in life face worse physical and mental health trajectories in their adolescent and adult years. Though there is ample evidence connecting these associations, the biological mechanism underlying these long-term effects remains poorly understood. A growing body of evidence indicates that exposure to adversity early in life can lead to long lasting changes in a number of systems, including the immune system, and brain structure and function. Exposure to adverse environments may also be accompanied with changes in the epigenome, in particular DNA methylation. Studies have also shown that the timing and diversity of adverse events, as well as sex differences, may impact the severity of these associations. Utilizing longitudinal survey data and DNA samples from the Quebec Longitudinal Study of Child Development (QLSCD), the link between early adversity, the timing of adversity, and DNA methylation in adolescence, as well as sex specific outcomes are investigated. Here, early adversity is defined by both a cumulative adversity score, derived from multiple indicators of socioeconomic and psychosocial stress repeatedly and prospectively collected from infancy to 15 years of age, as well as a perinatal adversity score, utilizing adversity measures collected at 5 months of age. In this study, DNA methylation is measured using the EpiStress Score, a polyepigentic score created from 24 CpG sites specific to glucocorticoid exposure, which acts as a measure of stress exposure. Associations between the EpiStress Score and each adversity measure were initially considered separately, however, it became evident that both adversity measures were required to see any significant associations. Moreover, sex differences interacted with our adversity measures, resulting in a decomposition of our variables by sex for further analysis. Our results of these associations implicated sex and adversity timing as key differentiators in the way adversity impacts adolescents' epigenomes.

SYSTEMATIC MAPPING OF NUCLEAR DOMAIN-ASSOCIATED TRANSCRIPTS REVEALS SPECKLES AND LAMINA AS HUBS OF FUNCTIONALLY DISTINCT POPULATIONS OF RETAINED INTRONS

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The nucleus is highly compartmentalized through the formation of distinct classes of non-membranous domains, yet the composition and function of many of these structures is not well understood. Using APEX2-mediated proximity labelling and RNA sequencing, we surveyed transcripts associated with nuclear speckles, several additional domains, and the lamina. Remarkably, speckles and lamina are associated with distinct classes of retained introns enriched in genes that function in RNA processing, translation, and the cell cycle. In contrast to the lamina-proximal introns, retained introns associated with speckles are relatively short, GC-rich, and enriched for functional sites of RNA binding proteins that are concentrated in these domains. They are also highly differentially regulated across diverse cellular contexts, including the cell cycle. Our study thus provides a resource of nuclear domain-associated transcripts and further reveals speckles and lamina as hubs of distinct populations of retained introns linked to gene regulation and cell cycle progression.

HIGH-THROUGHPUT EXPRESSION AND DNA METHYLATION SCREENING IN HUMAN CELLS AFTER BORRELIA BURGDORFERI INFECTION

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Tick-borne diseases such as Lyme disease, which is caused by *Borrelia* species spirochaete bacteria affects both humans and animals and is increasing in frequency globally. The interaction between host, tick and pathogen is multifaceted and the role of epigenetic processes remain largely unexplored. Environmental factors such as bacteria are known to shape the host epigenetic landscape; the epigenetic changes in host-pathogen interactions are poorly understood and remain to be investigated in tick-borne diseases. The acquisition and analysis of high-throughput data allows the study of gene expression and its potential control by DNA methylation. Therefore, we investigated the effect of *Borrelia burgdorferi* strain B31 for 72 hours on human primary cells (HUVEC) and a cell line (HEK-293). Both RNA and DNA were extracted from cells and subjected to RNA-seq and Enzymatic-Methyl-seq (EM-seq). Trimmed reads served as input into *Salmon*, whereas *Tximeta* and *DESeq2* in R were applied for further RNA-seq

data analysis. Illumina BaseSpace *MethylKit* App was used to analyze EM-seq data testing various settings. Differentially expressed pathways in humans were identified with the R*eactome* database. RNA-seq revealed fewer significantly differentially expressed genes in the HEK-293 (8) compared to HUVEC (69). Top pathways enriched for HEK-293 were Pre-NOTCH processing in Golgi, amyloid fiber formation, and metabolism of lipids. In HUVEC cytokine and other immune response signaling were identified as important pathways. Internal controls and quality parameters of EM-seq were satisfied. However, no significant methylation changes were detected in cells after *Borrelia burgdorferi* infection compared to uninfected cells, even using variable stringency. This indicates that *Borrelia burgdorferi* strain B31 does not affect host cell methylation patterns. Other epigenetic parameters remain to be investigated. This is the first study to measure transcriptomic changes after 72 hours of *Borrelia burgdorferi* exposure in Lyme disease model cells. The cell-dependent specific response will contribute to unravelling the pathology of Lyme disease.

A COMPENDIUM OF UNIFORMLY PROCESSED PUBLIC MOUSE HEMATOPOIETIC RNA-SEQ DATA FOR DATA MINING.

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Since its introduction more than a decade ago, RNA-seq technology has evolved into the gold standard for genome-wide gene expression quantification. In recent years, most researchers choose to share their raw sequence data, including RNA-seq reads, via the Sequence Read Archive (SRA) and other public repositories. In addition, Gene Expression Omnibus (GEO) provides experimental details on how the submitted data was generated. While together, GEO and SRA hold a great potential for large-scale data exploration, in order to make datasets generated in multiple experiments comparable, they need to be processed in a uniform way. Therefore, RNA-seq reads need to be aligned to the same reference, using the exact same strategy, gene annotation model and method of expression quantification. Additionally, such a comprehensive analysis should involve quality control (QC) steps to harmonize and allow for potential filtering of the data.

Hematopoiesis has been studied extensively as a model of cellular differentiation, which yielded a large volume of transcriptomic and epigenetic data generated from multiple cell types. In order to provide a uniformly processed and large repertoire of public RNA-seq data from mouse hematopoietic cells, we downloaded 183 public RNA-seq datasets, spanning a decade of experimental studies that were conducted by different laboratories around the world. Next, we QC'ed and uniformly processed this data, converting it from raw sequence reads to Transcripts Per Million (TPM) for all protein coding genes annotated in Ensembl v102 using the STAR aligner.

Currently, researchers upload their metadata and experimental information to the GEO database in a free format, which varies significantly. Therefore, harmonization of metadata was one of the corner stones of this work. We manually extracted metadata for each individual data sample within our compendium (n=1403), often frequenting the methods sections of the original publications. We focused on the metadata categories we deemed important for the analysis and comparison of data stemming from different experiments, including mouse age and sex, RNA isolation- and library construction method, sequencing technology and protocol (single- or paired-end), read length, and most importantly, comprehensive information on all cell surface markers that were used to isolate hematopoietic cell fractions. We only considered data from wild type C57BL/6 (or closely related) mice under normal, physiological conditions.

In sum, we provide a large, quality-controlled, and uniformly processed gene expression atlas of healthy mouse hematopoiesis that comprises both TPM- and corresponding metadata, which currently comprises 1403 individual RNA-seq data sets from 183 GEO submissions and >100 cell types, ranging from the earliest progenitors to most mature cells. All data was uploaded to the Python-enabled 'sqllite' database in a format that is convenient for data mining, e.g., for in-depth comparisons between transcriptomes, cell types, and cell populations, and to assess experimental biases.

SEXUAL EPIGENETICS – FROM PROFILES TO PATHWAYS

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Sexual reproduction is one of the most important processes for almost every form of life: it ensures genetically diverse offspring and, thus, survival of species in our ever-changing environment. Except for a select number of model organisms, however, little is known about sex determination in many organisms.

Classic understanding explains sex determination as either genetic or environmental. In recent years, evidence has been accumulating for more nuanced mechanisms, indicating key roles for epigenetic paths in sex determination in a growing number of species. For example, our recent work using high-resolution whole genome DNA methylation profiles combined with state-of-the-art machine learning identified the key regulator of sex in a common economically important plant. The uncovered mechanism involves a classical hormone pathway

and the female-specific expression of the key regulator. In male individuals, this central regulator is silenced via RNA-directed DNA methylation. Follow-up work provided functional proof and the identification of the underlying molecular pathways leading to sex-differential DNA methylation and sex expression. Analysis of the full developmental path from stem cell-like progenitors (reproductive meristems) to full reproductive structure indicated successive waves of chromatin-alteration to co-ordinate gene expression and ensure correct spatial and temporal development. Parallels to other organisms are drawn. Controlling such a fundamental biological process such as sex determination highlights the tremendous potential of epigenetic mechanisms in development and disease.

INVESTIGATING THE ROLE OF THE ANAPHASE PROMOTING COMPLEX IN DNA REPAIR PATHWAYS

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The Anaphase Promoting Complex (APC) is an evolutionarily conserved multi subunit protein complex that functions as a ubiquitin E3 ligase, targeting selected proteins for degradation in synchrony with the cell cycle and ensuring maintenance of genomic stability, regulated cell-cycle progression and longevity. The DNA damage arising within aging cells and with APC dysfunction is poorly understood, as it is not known whether single stranded (*ss)* or double stranded (*ds)* damage accumulates, nor if APC activity is required to repair one, or both. We propose to (semi) quantitate the *ss*- and *ds*-DNA damage in old cells, and those with APC mutations, and to also determine if *in vivo* APC activation can reverse or delay this damage. We will chemically induce *ss*- and *ds*-DNA damage in WT and APC-deficient cells and measure survivability as an indirect measure of APC-dependent repair. Results so far suggest that *dsDNA* damage accumulates in APC mutant strains and older cells, when compared to WT and younger cell populations. Our overall goal is to understand downstream consequences of altered APC activity on DNA damage and repair, and investigate the underlying mechanisms involved. These experiments may reveal novel cellular pathways that could be targeted for therapeutic interventions to minimize DNA damage or enhance healthy aging.

MECHANISM OF MITOTIC BOOKMARKING AND TRANSCRIPTIONAL MEMORY IN STEM CELLS.

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During multi-cellular development, a single zygote gives rise to all cell types of the body through successive rounds of cell division and differentiation. A fundamental property of the remarkable process is the ability of cell to change (plasticity) or maintain (memory) their state. Maintenance of the cell state means the faithful transmission of the transcription program throughout cell division (mitosis), while differentiation requires a reprogramming of the transcription profile from stem cell state to the differentiated state. However, a mechanism governing maintenance of cell identity during cell division is poorly understood. It was proposed that specific binding of transcription factor to mitotic DNA “bookmarks” genes for reactivation following cell division.

The goal of this project is to elucidate the molecular determinants for targeting or exclusion of TFs mitotic DNA. Using genomic approach, we mapped the binding of transcription factors Sox2 and HSF2 on the mitotic chromosomes. Furthermore, we investigated cellular localization of transcription factors using live-cell imaging. Surprisingly we found a that while Sox2 was detected on mitotic DNA using both genomic and imaging approach, the HSF2 showed a strong mitotic binding using genomics, but was not visible on the mitotic DNA by imaging. These results suggest the different modes of mitotic bookmarking by transcription factors.

DEFINING THE ROLE OF ATRX DURING FOREBRAIN DEVELOPMENT

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Aberrant epigenetic regulation is now recognized as a common cause of neurodevelopmental disorders, yet many syndromes remain poorly understood. Mutations in the *ATRX* gene, an ATP-dependent chromatin remodeling protein cause the ATR-X syndrome. Biochemical studies and characterization of *Atrx*-null mice have shown it is critical for the expansion of the neuroprogenitor pool. Studies in the retina identified a post-mitotic role for *Atrx* as bipolar neurons showing impaired axonal targeting and non-cell-autonomous effects on the survival of interconnected neurons. Recently, *Atrx* inactivation in forebrain pyramidal neurons showed synaptic ultrastructural defects and male-specific memory deficits. While these studies have helped elucidate *Atrx* function, individually they do not represent strong models of the ATR-X syndrome. Here, we describe the generation and characterization of *Atrx*EcKO mice using the *Emx1*-Cre driver line. This Cre driver was chosen due to its limited effect on progenitor expansion yet widespread activity in developing excitatory neurons of the cortex and hippocampus.

*Atrx*EcKO mice survive to adulthood, had reduced body weight, and lacked *Atrx* expression in the cortex and hippocampus (HPC). Developmental studies revealed hyperactivity, seizure-like episodes and, in some cases, self-injurious behavior that are features of the human disorder. Examination of the adult HPC showed a ~40% reduction in overall size and

disorganization of the neuronal and molecular layer in all subregions that was most striking in the dentate gyrus (DG). Within the cortex, a thinner corpus callosum and reduction in myelin density was observed but cortical thickness and lamination were normal. RNAseq analysis of dissected HPC tissue revealed 656 up- and 717 down-regulated transcripts (Log2FC ± 0.25; q-val, >0.05). Among those selected for further analysis were *Neurog2*, *Ntng1* and *Ntng2*. *Neurog2* is important for neurogenesis and migration, and we observed alterations in the proportion of Tbr2+ progenitors and migration within the 3 matrices of the DG migratory pathway. IF-staining demonstrated almost undetectable levels of presynaptic netrin-G1 and netrin-G2 in HPC, indicative of perturbed axonal guidance with resulting loss of lamina-specific termination of hippocampal circuits.

Collectively, the defects characterized in the HPC and DG, combined with the behavior abnormalities and partial agenesis of the corpus callosum, draw us to conclude that the *Atrx*EcKO mice replicate many features of the human disease and represent a good model of the ATR-X syndrome.

AN EPIGENOMICS QUALITY CONTROL STUDY TO ASSESS GENOME-WIDE METHYLOME SEQUENCING DATA

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Within the International Human Epigenome Consortium (IHEC), various research and sequencing centers have participated in generating a large collection of human epigenome reference maps from a comprehensive set of tissue and cell types relevant to health and disease. IHEC complete epigenomes consist of ChIP-seq, WGBS-seq and RNA-seq data. To coordinate the diverse IHEC members, the Assay Standards Working Group has defined protocols, Quality Control (QC) metrics and containerized bioinformatics pipelines for each assay type. This high-quality epigenomics data resource generated world-wide provides the basis of integrative analyses and comprehensive data assessments. The primary focus of this study is the IHEC WGBS-seq dataset which spans 513 reference epigenomes generated by 9 IHEC sequencing centers. This data has been uniformly analyzed using the GemBS bioinformatics pipeline which combines a read aligner and a methylation caller. The six core QC metrics defined by the Assay Standards Working Group (mapping efficiency, proportion of uniquely mapped reads without replicates and with a mapping quality score >10, average coverage per base, median CpG coverage, bisulfite conversion rate and GC bias) constitute the basis of this integrative analysis. Data quality was examined across this large collection of IHEC methylomes using the IHEC recommended standard threshold of 30X coverage and by leveraging the different number of replicates, sequencing methods (single-end, paired-end, different read lengths) and library construction protocols (standard WGBS, PBAT/PBAL).Our results reveal an explicit range for every core QC metric across the IHEC DNA methylation datasets and illustrate the high quality of the data generated as part of IHEC. They also show the importance of the availability of metadata specifications for the downstream integrative analyses.In conclusion, this study highlights the breadth of IHEC high resolution human DNA methylomes and provides a QC analysis and QC summary that will serve as a guide and resource for researchers in their downstream analyses in order to accelerate human epigenomics data discovery.

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). Our previous studies found that long-terminal-repeat retrotransposons (LTRs) transcriptionally active in oocytes are responsible for the wide differences in DNAme patterns in different species. We further showed that gene-body deposition of H3K36me3 by SETD2 guides *de novo* DNAme in oocytes, through recruitment of the DNMT3A-DNMT3L complex to the H3K36me3-marked regions. We showed that murine-specific LTRs upstream of the imprinted genes *Slc38A4* and *Impact* are active in oocytes, are marked by promoter-associated H3K4me3 and followed by downstream enrichment of gene-body H3K36me3. Together with PolII ChIP-seq, RNA-seq data and DNAme data, our results suggest that both genes are transcribed from the alternative LTR promoters in fully grown oocytes (GVOs), which leads to acquisition of DNAme at their promoter igDMR. We generated knock-out alleles for both LTRs and showed the maternal imprints of both *Slc38A4* and *Impact* were removed, and hence implicated the role of LTR elements in the imprinting. 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 currently conducting gain-of-function studies by generating a mouse line containing an oocyte-active LTR inserted upstream of a non-imprinted gene. *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. Mouse ESCs with the correct insertion allele have been obtained. On successfully establishing a knock-in mouse line, we will study transcription-

coupled *de novo* DNAme and imprinting signatures, such as DNAme, ChIP-seq on H3K4me3, H3K36me3, and PolII, and transition in the allelic expression based on SNP analysis. Our design also introduces *lox*P sites flanking the LTR insertion to test whether 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.

CELL-TYPE SPECIFIC OPEN CHROMATIN SIGNATURES IN THE BRAINS OF INDIVIDUALS DIAGNOSED WITH MAJOR DEPRESSIVE DISORDER AND DIED BY SUICIDE

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The genetic variants identified by Genome Wide Association Studies (GWAS) tend to be enriched in regions of the genome that do not produce functional proteins, referred to as the non-coding genome [1]. Indeed, for many psychiatric diseases, including major depressive disorder (MDD), GWAS single-nucleotide polymorphisms (SNPs) show substantial overlap with promoter and enhancer regions in a cell-type specific manner [2]. However, the manner in which the non-coding regions can alter gene expression patterns underlying disease phenotypes remains unclear. Chromatin accessibility of non-coding regions determines their activity in a cell-type specific manner [3]. Therefore, we will use a droplet-based Single-Nucleus Assay for Transposase-Accessible Chromatin sequencing (snATAC-seq) approach [4] to profile the prefrontal cortex of 44 individuals who during an episode of MDD had died by suicide and 44 sex- and age-matched psychiatrically healthy controls. We will capture hundreds of thousands of nuclei from the prefrontal cortex resulting in the largest snATAC-dataset in the brain to date. To mitigate batch-effects of capture and sex, we developed a machine learning approach that uses sex-specific chromatin features and 1000 genome common variants using Vireo [5] for in silico splitting of the multiplexed libraries at single-cell resolution. Top accessible features were computed for dimensionality reduction using latent semantic indexing (LSI) followed by graph-based clustering. Cell-types were annotated using the accessibility of promoters and enhancers of cell-type marker genes using Signac and Cicero, R packages [6,7]. Importantly, snATAC-seq data was integrated with snRNA-seq data [8] produced from the same subjects, to identify regulators of gene expression changes associated with depression phenotype. Our data showed co-accessible non-coding regulators of genes with distal enhancers showing cell-type specific patterning. Our preliminary analysis resulted in differentially open chromatin regions between cases and controls with enrichments for gene regulatory sites and transcription factor footprints in specific brain cell-types. In the excitatory neurons of MDD individuals, differentially closed chromatin sites are enriched for gene ontology terms including cognition, social behavior, and synaptic transmission, while differentially open regions are enriched for pathways that are known to regulate anti-depressant responses. Further, the transcription factor binding sites in the cortical cells of MDD individuals, such as for TWIST1 and CREB, have been previously reported to play causal roles in depression pathology [9]. We believe that profiling open chromatin across cell-types in phenotypic states will provide granularity to previously identified MDD associated SNPs and will elucidate the epi/genetic architecture underlying the etiopathogenesis of MDD.

HISTONE H3.3 K27M AND K36M MUTATIONS DE-REPRESS TRANSPOSABLE ELEMENTS THROUGH PERTURBATION OF ANTAGONISTIC CHROMATIN MARKS

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Histone H3.3 Lysine-to-Methionine substitutions K27M and K36M impair the deposition of opposing chromatin marks, H3K27me3/me2 and H3K36me3/me2. We show that these mutations induce hypotrophic and disorganized eyes in *Drosophila* eye primordia. Restriction of H3K27me3 spread in H3.3K27M and its redistribution in H3.3K36M result in transcriptional deregulation of PRC2-targeted eye development and of piRNA biogenesis genes including *krimp.* Notably, both mutants promote redistribution of H3K36me2 away from repetitive regions into active genes, which associates with retrotransposon de-repression in eye discs. Aberrant expression of *krimp* represses *LINE* retrotransposons but does not contribute to the eye phenotype. Depletion of H3K36me2 methyltransferase *ash1* in H3.3K27M, and of PRC2 component *E(z)* in H3.3K36M, restores the expression of eye developmental genes and normal eye growth, showing that redistribution of antagonistic marks contributes to K-to-M pathogenesis. Our results implicate a novel function for H3K36me2 while they showcase convergent downstream effects of oncohistones that target opposing epigenetic marks.

A CRISPR/CAS9 SCREENING STRATEGY TO IDENTIFY MODIFIERS OF THE VIRAL MIMICRY RESPONSE

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'Viral mimicry' is the induction of a cellular antiviral response triggered by endogenous nucleic acids rather than exogenous viral infection. Such nucleic acids are sensed by cytosolic pattern recognition receptors (PRRs) and interpreted by the cell as an infection, leading to activation of an antiviral signaling cascade that results in interferon (IFN) signaling and subsequent upregulation of interferon-stimulated genes (ISGs). While the downstream effects of ISGs are diverse and vary from direct induction of apoptosis to engagement of the adaptive immune system, the end result of viral mimicry is the culling of the 'infected' cell.

The viral mimicry response was first described as a mechanism-of-action behind the FDA-approved DNA demethylating agents azacytidine (AZA) and decitabine (DAC). By removing transcriptionally repressive DNA methylation, these compounds allow normally-silenced repetitive elements of the genome to be expressed as double-stranded RNA (dsRNA) species that trigger viral mimicry. Since then, a body of literature has emerged implicating viral mimicry as a common mechanism underlying many different therapies and drug targets. These targets include other epigenetic modifiers (e.g. LSD1, EZH2) as well as non-epigenetic targets such as cell cycle regulators (e.g. CDK4/6). The diversity of viral mimicry-inducing targets highlights the need for a systematic and discovery-based method to identify novel targets.

To accomplish this, we have developed and validated a viral mimicry cell reporter system for CRISPR/Cas9-based pooled library screening. This system is composed of a green fluorescent protein (GFP) reporter coupled to the interferon-stimulated response element (ISRE). Activation of the ISRE during interferon signaling thus also results in GFP expression, which can then be quantified by flow cytometry to identify the cells undergoing viral mimicry. To date, two preliminary screens in colorectal and lung cancer cell lines have been performed using this system in conjunction with a pooled single guide RNA (sgRNA) library targeting known epigenetic modifiers. These experiments have revealed two candidate hits, the histone methyltransferase SETDB1 and the lysine demethylase KDM5C, for further study.

EXPLORING THE EPIGENETIC LANDSCAPE OF CHEMORESISTANT TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, with earlier age of onset and worse prognosis. Although tumours initially respond to chemotherapy, there is a high rate of recurrence due to chemotherapy resistance, leading to poor overall survival. 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, where pharmacological inhibition of EZH2 induced activation of viral mimicry. Although the anti-tumoural effect of viral mimicry has been demonstrated in our taxol-resistant models as well as in other cancer types, it is currently unknown whether similar epigenetic vulnerabilities are present in TNBC harbouring resistance to different chemotherapeutic agents. To address this, we aimed to further study the chromatin variants underlying chemoresistance in other TNBC models. We hypothesize that viral mimicry evasion is a common mechanism of resistance and a potential avenue for therapy.

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). To investigate whether viral mimicry evasion is observed across all chemoresistant TNBC, we assessed gene expression for a panel of viral mimicry-related genes. We will identify chemoresistance-associated chromatin variants using a combination of Western blotting and LINE-1 ELISA to measure global histone and DNA methylation changes, respectively, and ATAC-seq to profile chromatin accessibility. Overall, our goal is to understand the epigenetic changes contributing to drug resistance to identify new vulnerabilities and therapeutic opportunities.

NAIVE HUMAN EMBRYONIC STEM CELLS CAN GIVE RISE TO CELLS WITH A TROPHOBLAST-LIKE TRANSCRIPTOME AND METHYLOME

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Human embryonic stem cells (hESCs) readily differentiate to somatic or germ lineages but have impaired ability to form extra-embryonic lineages such as placenta or yolk sac. Here, we demonstrate that naive hESCs can be converted into cells that exhibit the cellular and molecular phenotypes of human trophoblast stem cells (hTSCs) derived from human placenta or blastocyst. The resulting "transdifferentiated" hTSCs (tdhTSC) show activation of core placental genes, and the ability to differentiate to extravillous trophoblasts and syncytiotrophoblasts. Given the high heritability of DNA methylation across cell division and developmental transitions, as well as the distinctiveness of the placental methylome, we then compared the DNA methylation pattern of the tdhTSCs to that of parental hESCs and placentally derived hTSCs. The methylome of the tdhTSC is globally and locally similar to that

of placental hTSCs, including loss of methylation over the promoters of placental genes and gain of methylation over CpG islands specifically methylated in placenta. Modest differences are observed between transdifferentiated and placental hTSCs, most notably in the methylation status and expression of certain imprinted loci, which retain abnormalities that reflect the tdhTSC's transit through primed and naïve pluripotency. Collectively, these results suggest that naive hESCs can differentiate to extra-embryonic lineage and demonstrate a new way of modeling human trophoblast specification and placental methylome establishment.

PREDICTION OF HYPERTENSIVE DISORDERS IN PREGNANCY USING FIRST TRIMESTER CIRCULATING MICRORNAS

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Introduction: Gestational hypertension (GH) and preeclampsia (PE) are diagnosed in 5–7% of pregnancies and are associated to many adverse maternal and fetal outcomes with high morbidity and mortality. Although these conditions normally develop after the 20th week of pregnancy, accurate prediction is required for both prevention and optimal management. However, early biomarkers accurately predicting GH and PE before their onset are still lacking. microRNAs are small non-coding RNA sequences regulating numerous biological processes and secreted in blood from various organs including the placenta. We hypothesized that women who develop hypertensive disorders during pregnancy are characterized by a unique circulating microRNAs profile as early as the first trimester of pregnancy and that these microRNAs play a role in the development of GH and PE. Therefore, we sought to identify first trimester circulating microRNAs associated with GH and PE and the biological pathways they regulate as well as to assess their predictive value in both conditions. **Methods:** Using next-generation sequencing, we quantified first trimester plasma microRNAs from 28 GH, 22 PE and 385 normotensive women enrolled in Gen3G, a prospective pregnancy cohort. We identified differently abundant microRNAs using DESeq2 package and their targeted biological pathways using DIANA miRpath. We used stepwise logistic regression models on a training set (70% of the samples) to select the most promising microRNAs to predict GH and PE. Maternal age, gestational age and BMI at first visit were also considered in these models. The performance of the models was assessed on a test set (30% remaining samples) using the receiver operator characteristic (ROC) curve and the Youden index was set as the threshold to obtain the optimal sensitivity and specificity for each model. **Results:** A total of 28 (20 less and 8 more abundant) and 73 (38 less and 35 more abundant) microRNAs displayed differential abundance in women developing GH and PE respectively as compared to normotensive women ($p < 0.05$). These microRNAs were found to target genes regulating estrogen signaling, inflammation, hypoxia and angiogenesis related pathways (False discovery rate [FDR] adjusted $q < 0.05$). A final model which retained 6 microRNAs and gestational age at first visit predicted GH with an area under the ROC curve (AUC) of 0.803 (95%CI, 0.652-0.954), a sensitivity of 87.5% and a specificity of 70.4%. Another relatively simple model retained 6 other microRNAs in addition to first visit BMI and achieved an AUC of 0.748 (95%CI, 0.540-0.955), with a sensitivity of 83.3% and a specificity of 70.4% to predict PE. **Conclusions:** We have identified circulating microRNAs associated with GH and PE. These microRNAs regulate pathways linked to the pathophysiology of these conditions and hold promising results to improve the identification of women at high-risk before the onset of GH and PE.

SUBSTRATE H2B MEDIATED ACTIVATION OF DIMERIC E3 LIGASE UBR7 FOR DISCTINCT MODE OF UBIQUITIN TRANSFER

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Histone Ubiquitination, despite being key regulators of gene expression, DNA repair and epigenetic cross-talk, remains surprisingly less studied among all the various histone modifications. Inside the eukaryotic nucleosomes, histones H2A and H2B have been predominantly reported to be monoubiquitinated. In vertebrates, Histone H2B gets mono-ubiquitinated in the conserved Lys 120 (K120), corresponding to Lys-123 (K123) in Budding Yeast. In humans, H2B K120 monoubiquitination is linked with disruption of chromatin compaction and increased transcription and the loss of global levels of H2BK120Ub levels inside the cell is associated with Triple Negative Breast Cancer (TNBC) among many other different cancers. RNF20/40 heterodimer complex has been previously known to mediate H2B K120 monoubiquitination. We identified Ubiquitin Protein Ligase E3 Component N-Recognin 7 (UBR7) to be another novel H2BK120 monoubiquitin ligase which pairs with E2 conjugate UbcH6 for its E3 ligase function. In the current study, we show that atypical PHD zincfinger of UBR7 is essential for interaction with UbcH6 and concomitant transfer of Ubiquitin to substrate histone H2B. The PHD finger has been widely reported previously to operate as a reader of methylated, acetylated and unmodified histones, but here we have explored its role as a writer of histone post-transcriptional modifications. We have also identified the critical region of UbcH6 involved in this function and ascertained that

histone H2B C-terminal tail is necessary and sufficient for UBR7/UbcH6-mediated Ubiquitin transfer. Our work establishes that UBR7-PHD exists in solution as a dimer and this dimerization was found to be crucial in regulating E3 ubiquitin ligase function of UBR7, with dimer deficiency of UBR7 being implicated in cancer. In addition, we have compared the mode of ubiquitin transfer of UBR7 to RNF20, a previously reported H2BK120 ubiquitin ligase, and found that unlike RNF20, the UbcH6-Ubiquitin hydrolysis mediated by UBR7 requires substrate histone H2B association. Interestingly, the association of substrate H2B to UBR7 induces a conformational change in the E3 ligase which we have found to be critical for efficient ubiquitin transfer. No such conformational change is not observed in case of RNF20. Thus, the mechanism of ubiquitin transfer by the newly identified E3 ubiquitin ligase UBR7 is significantly different from that of RNF20.

ATXR5/6 FORMS ALTERNATIVE PROTEIN COMPLEXES WITH PCNA AND THE NUCLEOSOME CORE PARTICLE

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The proliferating cell nuclear antigen (PCNA) is a sliding clamp associated with DNA polymerases and servesas a binding platform for the recruitment of regulatory proteins linked to DNA damage repair, cell cycle regulation, and epigenetic signaling. The histone H3 lysine-27 (H3K27) mono-methyltransferase Arabidopsis trithorax-related protein 5/6 (ATXR5/6) associates with PCNA, and this interaction has been proposed to act as a key determinant controlling the reestablishment of H3K27 mono-methylation following replication. In this study, we provide biochemical evidence showing that PCNA inhibits ATXR6 enzymatic activity. The structureof the ATXR6 PCNA-interacting peptide (PIP) in complex with PCNA indicates that a trio of hydrophobic residues contributes to the binding of the enzyme to the sliding clamp. Finally, despite the presence of three PIP binding clefts, only two molecules of ATXR6 bind to PCNA likely enabling the recruitment of a third protein to the sliding clamp. Collectively, these results rule out the model wherein PCNA-bound ATXR6 actively reestablishes H3K27 mono-methylation following DNA replication and provides insights into the role of ATXR6 PIP motif in its interaction with PCNA.

TARGETING THE TRANSITION BETWEEN QUIESCENT AND ACTIVATED STEM CELLS IN MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most malignant brain tumour in children. Survival outcomes of MB are poor, and relapse is common. Our lab has published evidence of a cellular hierarchy in a mouse model of the sonic hedgehog subgroup medulloblastoma (SHH-MB), characterized by hyper-activated SHH pathway signalling, with a rare compartment of *Sox2*-expressing tumour stem cells capable of driving long-term tumour growth and relapse *in vivo*. Whereas most stem cells in MB tumours are quiescent, a fraction of them express *Mki67*, a proliferation marker. A major unanswered question in the medulloblastoma lineage hierarchy is how quiescent, self-renewing stem cells transition into the rapidly cycling progenitor cells to generate the tumour bulk. The switch from stem cell quiescence to activation could be a pivotal step in tumorigenesis and a key therapeutic window. Applying whole-genome and functional approaches to the tumour subsets, we determine that *Olig2* is a regulator of stem cell activation and emergence from quiescence in Shh-MB tumours.

Olig2 is frequently over-expressed in gliomas, however, its role in SHH MB tumour initiation is not well understood. We observed decreased proliferation and sphere-forming capacity in CRISPR-mediated *Olig2* knockout mouse MB tumour cells cultured in stem cell-enriching media, and also report a significant delay in their ability to form tumours compared to control in a subcutaneous allograft assay. Inhibiting *Olig2* function *in vitro* decreases cellular proliferation and enriches for a more potent sphere-forming stem cell population. Finally, using a small molecule of inhibitor of *Olig2* function in a transgenic mouse model of SHH-MB, we prevent tumour initiation as well as relapse post-debulking with a smoothened inhibitor. Taken together, these data suggest that *Olig2* plays a key role in promoting the stem cell proliferation crucial for tumour development. Our results lead to a deepened understanding of tumour initiation in SHH MB and reveal a potential therapeutic target.

DNA METHYLATION BIOMARKERS OF CARCINOGENIC EVENTS IN BENZO[A]PYRENE TREATED HAMSTER FETAL CELLS.

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Background. Many assays can identify genotoxic carcinogens but no validated short-term tests exist to identify non-genotoxic carcinogens (NGTxCs). Currently, the only means to classify NGTxCs include long-duration animal studies, but the U.S. Environmental Protection Agency will phase out such bioassays in 2035. Consequently, regulatory agencies need new methods

to detect carcinogens. The Syrian Hamster Fetal Cell Transformation assay (SH-CTA) is a 7-day *in vitro* test, that evaluates cellular morphological transformation (MT) to predict chemical carcinogenicity (including NGTxC), and reduces animal use. However, the subjective assessment of MT and its mechanistic relevance to human carcinogenicity have been questioned.

Objectives: 1) To investigate the chronology of DNA methylation changes from primary cells to sustained proliferation. 2) To identify DNA methylation markers to complement or improve CTAs.

Methods. The SH-CTA was performed using a known carcinogen (benzo[*a*]pyrene) and we collected cultures with varying status with regard to treatment, duration, and cellular transformation steps from primary cells to senescence bypass and sustained proliferation toward cancer. DNA methylation was analysed by reduced representation bisulfite sequencing and differentially methylated regions (DMRs) were confirmed by bisulfite pyrosequencing (Pyro).

Results. As expected, some groups of cells showed morphological transformation at day-7. These cultures were maintained longer until they displayed a period of growth arrest (senescence), but from which some cells emerged (senescence bypass) and displayed sustained proliferation (SP) as an essential key characteristic of cancer cells. Minimal changes in DNA methylation were observed at day-7. Profound DNA methylation changes arose during cellular senescence and bypass stages that were preserved through SP. A set of important genes that maintained DMRs from senescence until SP (e.g.: *Pou4f1*, *Aifm3*, *B3galnt2*, *Bhlhe22*, *Gja8*, *Klf17*, and *Line-1*) were used to confirm by Pyro their reproducibility across multiple clones with different passage numbers and obtained from other laboratories.

Conclusion. These DNA methylation changes could serve as biomarkers to enhance objectivity and mechanistic understanding when using CTAs to predict chemical carcinogenicity. This work has the potential to improve testing strategies for the identification of all carcinogens, including NGTxC. Funded by Health Canada's Chemicals Management Plan.

MODULATION OF MICROGLIAL TRANSCRIPTOMIC AND EPIGENOMIC PROGRAMS AFTER SYSTEMIC PRO-INFLAMMATORY STIMULUS

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Background
Systemic immune response syndrome (SIRS) is a critical medical condition caused by diverse infections, including several bacterial infections and potentially COVID-19, and associated to long-term complications. SIRS is accompanied by a functional breakdown of multiple organs, including the brain. This results in a neuroinflammatory response orchestrated by microglia, the brain's resident macrophage. However, the transcriptional and epigenetic mechanisms underlying this response remain under-characterized.

Methods
To study these mechanisms, we used a systemic inflammation murine model in which SIRS is induced by injection of bacterial lipopolysaccharides (LPS). At several time points following LPS injection, microglia were extracted to perform massively parallel sequencing. RNA-seq was used to evaluate the transcriptome and was coupled with ATAC-seq to find transcription factor motifs at associated promoter regions. ChIP-seq targeting histone mark H3K27ac allowed assessment of activity at distant genomic regulatory elements.

Results
Results show that microglia's transcriptomic and epigenomic activities during neuroinflammatory response follow different dynamic profiles. RNA-seq data indicate a wave of pro-inflammatory gene transcription starting 3h after induction of SIRS. This initial wave correlates with enrichment of Nfy, Sp/Klf and Pu.1 motifs at associated promoters. It is followed by increased activity at distant genomic regulatory regions that is regulated by transcription factors Nf-κB, AP-1 and Irf. Furthermore, this whole process leads to increased microglial proliferation with a distinct transcriptional profile from that of other proliferating conditions.

Conclusions
These results define the progression of intrinsically connected transcriptional and epigenetic programs of microglia during SIRS. As such, they provide potential therapeutic targets that could be exploited to lessen brain sequelae in SIRS.

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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Activity from the DNMT3A enzyme 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. In mice, complete lack of DNMT3A enzyme is

lethal, whereas *Dnmt3a*$^{+/-}$ animals are normal and fertile. In humans, DNMT3A-deficiency in embryonic stem cells leads to loss of DNA methylation in a lineage and tissue specific manner as cells differentiate. Rare heterozygous mutations in functional DNMT3A domains cause an overgrowth intellectual disability syndrome. Currently, we do not know how functional mutations in the human DNMT3A protein 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 (**iPS**) cell lines, each carrying a single mutation in the functional methyltransferase domain of DNMT3A and reprogrammed these cells into neural progenitors. The mutated iPS cells expressed pluripotency markers and showed good differentiation ability into neural progenitors, comparable to control cells. We generated RNA-seq profiles from iPS and neural progenitor cells and performed bioinformatics analyses. Our results show that alteration in DNMT3A function leads to abnormal expression in iPS cells (n=1333 genes; Higher expression n=475, Lower expression n=858) compared to controls. Following cellular specification of *DNMT3A* mutant iPS cells into neural progenitors, we observed a significant increase in altered transcriptomic expression (n=3936 genes; Higher expression n=1922, Lower expression n=2014) in comparison to controls. While the top enriched genes in *DNMT3A* mutant iPS cells are associated to development and neurodevelopment, the top enriched genes in *DNMT3A* mutant neural progenitors are associated to embryogenesis and cell fate decisions. These results show that although they do not affect the reprogramming process for iPS cell generation, heterozygous DNMT3A mutations in the methyltransferase domain lead to altered gene expression profiles that are exacerbated during differentiation into neural progenitors.

Further analyses are needed to understand the mechanisms by which pathogenic DNMT3A alters gene regulation, and how this can affect lineage specification of neural progenitor cells.

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, decrease levels of repressive histone modification H3K9me3 associated with imprinting loci, increase levels of active mark H3K4me3 associated with actively transcribed genes, increase in H3K27ac associated with open chromatin, and increase in gene expression. My current results show that when I target such imprinted region in mESC using non-allele-specific epigenome editing systems I can modify the methylation status of the regulatory region and directly impact gene expression.

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 a CRISPR-based epigenome editing system with a deactivated Cas9 (dCas9) nuclease fused to either the catalytic domain of the Ten-Eleven Translocation 1 protein (TET1) or the catalytic domain of DNMT3A, and guide RNAs (gRNA) targeting specific 20bp DNA sequences. This system in combination with mESCs derived from the backcross of two different strains (B6xCAST7) allows us to identify single nuclear polymorphisms (SNPs) and design gRNAs to accomplish the allele-specificity of the system.

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.

LONG TERM CULTURE OF HUMAN INTESTINAL ORGANOIDS LEADS TO GLOBAL DNA METHYLATION CHANGES

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Organoids are a powerful tool to model aspects of development, health and disease. A necessary aspect of organoid models is the expansion of cultures *in-vitro* through several rounds of passaging. This is of potential concern as high passaging of cell cultures has been shown to affect cellular function. We have generated genome wide DNA methylation (DNAm) profiles from 80 human intestinal organoids. Our analyses revealed major effects of passage on DNAm with significant changes at 61,337 CpGs. High passage organoids were globally hypomethylated and locally hypermethylated, with greater variability in DNAm with increasing passage. Importantly, we were able to validate observed passage effects in 76 publicly available organoids, including healthy gut organoids and organoids generated from malignant tissues including pancreas, rectum, stomach, and lung. Together, our findings suggest a major impact of prolonged culturing on global organoid DNAm profiles, highlighting the importance of considering passage in organoid experiments.

TEMPORARY INACTIVATION OF DNMT1 INDUCES PERMANENT EPIGENETIC ERRORS IN EARLY EMBRYONIC CELLS

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The maintenance of DNA methylation (DNAm) by DNA methyltransferase 1 (DNMT1) is particularly important for regulating cellular differentiation, the process through which a stem cell undergoes specialization. During differentiation, DNAm patterns are dynamic, but certain profiles must be maintained by DNMT1 as a form of epigenetic memory, contributing to lineage classification and quality control of stem cells. This project aims to investigate the epigenetic consequences in promoter regions if DNMT1 is temporarily inactivated and determine the impact on differentiation potential towards germ layers. Since we know that epigenetic mechanisms rarely act alone and instead interact with each other to regulate the genome, we suspect that deregulation of DNMT1 will cause ricochet effects on other mechanisms such as histone modifications.

We used a mouse embryonic stem cell (mESC) model in which a doxycycline controllable cassette was added to the *Dnmt1* promoter; *Dnmt1* is inactivated upon doxycycline treatment and reactivated when doxycycline is removed. We then measured changes in DNAm by RRBS, histone modifications (H3K4me1, H3K4me3, H3K27me3, H3K27ac, H3K9me3) by ChIP-seq and gene expression (RNA-seq).

We show that temporary *Dnmt1* inactivation causes permanent errors in DNAm and histone modifications within promoters of developmental importance, with histone modifications being surprisingly far more affected than DNAm. This could be explained by the fact that epigenetic regulators, including many histone modifiers, also become temporarily up or downregulated when *Dnmt1* is transiently absent; Some even remain deregulated upon *Dnmt1* reactivation (e.g., Lysine (K) methyltransferases *Kdm4b*, *Kdm5d*, *Kdm7a*). Poised chromatin, essential for maintaining pluripotency, is significantly altered hinting that stem cell quality and differentiation potential could be affected. Upon differentiation to neuroectoderm, hundreds of genes become dysregulated, many of which are involved in neurodevelopment but more strikingly, 5 early endodermal transcription factors and 3 transdifferentiation factors become upregulated suggesting that these cells may not be able to properly follow their developmental path.

This project will demonstrate the importance of maintaining specific epigenetic profiles in embryonic stem cells as to ensure proper regulation of early development and show how temporarily disturbing DNAm maintenance induces a butterfly effect on histone modifications and gene expression. In the future, we will use epigenome editing to correct these errors in early embryonic cells.

ZBTB7B: A NOVEL REGULATOR OF MONONUCLEAR PHAGOCYTES

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ZBTB7B (also known as ThPOK) is an essential transcription factor for the development of T helper (Th) lymphocytes. Recently, our lab participated in a study reporting a severe susceptibility to mycobacterial infection in mice carrying a loss-of-function mutation in *Zbtb7b*. As protection against mycobacterial infection in mice is primarily regulated by the intricate interplay between Th1 cells and the mononuclear phagocytes from the innate immune system, we postulated that the severe susceptibility to mycobacterial infection was not due to the solo absence of Th populations in *Zbtb7b* mutated mice. At the first step, high-dimensional flow cytometry was carried out to examine the distribution of immune cells in the spleen of *Zbtb7b* knockout (*Zbtb7b*-/-) mice. The numbers of CD4+ plasmacytoid dendritic cells (pDC), were found to be reduced by approximately 60% compared to wild-type littermate. In a follow-up *in-vivo* challenge study, intradermal infection with *Leishmania major* resulted in significantly larger lesions and higher parasite load in *Zbtb7b*-/- mice when compared to wild-type controls, suggesting a functional defect of the macrophages, which are the main host cells for *Leishmania*. RNA-seq profiling of bone marrow-derived macrophages (BMDM) showed that *Zbtb7b* is expressed and ChIP-seq revealed that ZBTB7B is recruited to thousands of genomics binding sites. Preliminary results showed 4240 BMDM-specific

binding sites for ZBTB7B in comparison to Th lymphocytes. Of note, ZBTB7B binds the *fms*-intronic regulatory element of *Csf1r*, which is a highly conserved super-enhancer known to have a pivotal role in developing tissue-resident macrophages. In addition, *Zbtb7b*-/- BMDMs challenged with bacillus Calmette-Guérin (BCG) produced a significantly lower amount of nitric oxide. Overall, the results of this ongoing study highlight a role for ZBTB7B in the development of pDCs and the regulation of macrophage response to intracellular pathogens. Further studies are ongoing to elucidate how ZBTB7B regulates the number and function of pDCs, and its role ZBTB7B in the epigenetic rewiring of mononuclear phagocytes and the extent of its effect on protection against intracellular pathogens.

A SYSTEM BIOLOGY APPROACH IDENTIFIES CANDIDATE DRUGS TO REDUCE MORTALITY IN SEVERELY ILL COVID-19 PATIENTS

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Introduction: Coronavirus Disease 2019 (COVID-19) is characterized by heterogeneous clinical outcomes of infection ranging from absence of clinical symptoms to death. Despite the availability of efficacious vaccines, COVID-19 lacks effective drugs to treat hospitalized patients. To address this therapeutic shortcoming, we applied a system biology approach to study critically-ill patients for whom life-saving treatment remains an urgent unmet need.

Methods: We enrolled patients with the same severe clinical WHO ordinal scale (OS=7) at hospital admission and followed them for 15 days. Of the seven enrolled patients, three died of COVID-19 while four recovered and were discharged. We retrospectively contrasted the transcriptomic and epigenetic landscape of critically-ill patients who recovered (Alive) with those who perished (Deceased). Pathways significantly different between Deceased and Alive groups were used to identify drugs for repurposing. We used scRNAseq to integrate 105,851 cells, ATACseq to quantify chromatin accessibility of 13398 regions and performed whole genome bisulfite sequencing (wgBS) to test DNA methylation in 26,840,057 CpG loci.

Results: When patients were retrospectively grouped according to “Deceased” or “Alive”, we failed to detect differences in PBMC proportions at hospital admission, however, we observed strong transcriptional changes. In prospectively deceased patients, transcriptomic analysis identified CD14+ monocytes as high responder cells. Of the 13,398 chromatin regions tested for CD14+ monocytes, 959 were differential accessible chromatin (DAC) at admission and 407 were DAC at follow-up. A total of 54.5% of DAC regions at admission had sustained significance at the follow-up, including DAC located at COVID-19 GWAS loci. The wgBS detected 6,259 differentially methylated regions at admission, of which ~40% were conserved during disease progression. Following data integration, we observed a high level of consistency between the Gene Ontology (GO) terms and pathways between different assays. Interferon and splicing-related pathways were detected by Deceased vs Alive differences in gene expression, chromatin accessibility and DNA methylation. Splicing-related terms were the most significant GO detected at follow-up. Searching for existing drugs interacting with the GO/Pathways detected by at least two assays resulted in the identification of three candidate drugs, namely Tacrolimus, Zotatifin and Nintedanib.

Conclusion: At the time of hospital admission clinically equivalent patients displayed significant different monocyte epigenetic and transcriptomic attributes between those who would survive and those who would die from COVID-19. We have identified key host response pathways over activated at hospitalization by patients who would not survive. The identified pathways are targetable by three candidate repurposing drugs that can be evaluated to reduce mortality of critically ill COVID-19 patients.

CHARACTERIZING THE CIS-REGULATORY ELEMENTS RESPONSIBLE FOR REGULATING SFMBT2 & TROPHECTODERM SPECIFIC ENHANCERS

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Extraembryonic development is a remarkably important aspect of an organism’s life yet has been overshadowed by research in embryonic tissues. Placental formation and development has been implicated in numerous defects to embryonic development which can cause altered metabolic and behavioral phenotypes leading to lifelong complications. Comparatively less is known about the transcriptional regulatory network in the placenta and within the Trophoblast Stem Cells (TSCs), the progenitor cells that form the placenta, compared to what is known in embryonic stem cells. TSCs have a unique repertoire of transcription factors that act upon cell-type specific enhancers to in turn regulate trophectoderm-specific genes. An example of these trophectoderm-specific genes is *Sfmbt2*, a maternally imprinted, predicted polycolmb group protein responsible for regulating key genes in TSC maintenance. Paternal deletion of *Sfmbt2* is embryonic lethal in mice due to a failure in placental development and Sfmbt2 expression is required for TSC self-renewal. Our goal is to identify the cis-regulatory elements and trophectoderm-specific factors are responsible for regulating *Sfmbt2* in TSCs, and in the developing placenta. By employing allele specific enhanced chromosome conformation capture (e4C) in TSC, we determined a region of DNA 65kb upstream of *Sfmbt2* interacts

preferentially with the active, paternal *Sfmbt2* promoter, while the inactive maternal promoter interacts with a heterochromatic region of DNA downstream of *Sfmbt2*. The upstream interacting region is marked by histone 3, lysine 27 acetylation and contains two potential enhancers (UR1 & 2). Dual-luciferase assays on the two putative TSC enhancers showed significant enhancer activity in UR1 while UR2 did not appear to have significant activity. Mutations of predicted transcription factor binding sites for *Cdx2*, *Tead4*, *Tfap2c*, and *ELF5* caused a significant reduction in enhancer activity comparable to that of the wild type enhancer. Finally, CRISPR-Cas9 mediated deletion of the UR1-UR2 region in TSCs resulted in a significant decrease in *Sfmbt2* expression, as well as genes upstream of *Sfmbt2* that may affect the ability of TSCs to differentiate properly. *In vivo* mouse work is currently underway to determine whether a deletion of these enhancers causes any phenotype variation during mouse development.

PREDICTING METADATA FROM DATA: VALIDATING OVER 15,200 CHIP-SEQ PUBLIC DATASETS

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Over the last decade, major initiatives such as the International Human Epigenome Consortium (IHEC) that includes ENCODE 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 such as GEO (Gene Expression Omnibus). Since the metadata hosted in GEO has no standardization, initiatives such as ChIP-Atlas and NGS-QC have tried to harmonize the GEO metadata. However, 35% of the targets are still missing or have discrepant information. Furthermore, about a third of the metadata reinterpretation is different between these initiatives. Based on our previous works developing the epiGeEC (epigenomic Efficient Correlator) tool, we hypothesize that it could be possible to use the epigenomic data to predict their metadata.

To test this idea, we used the processed data generated by the different consortia stored on the IHEC data portal to develop and train EpiLaP (Epigenomic Labeling Predictor) v0.1, a neural network composed of 3,000 nodes fully connected having accuracy and precision >98%. We tested this preliminary version of EpiLaP on the harmonized ChIP-seq EpiAtlas data generated by the IHEC Integrative Analysis working group. EpiLaP detected few potential sample inversions (prediction value >0.9) supported by correlations from epiGeEC and visual inspections through a genome browser. Since the harmonization pipeline used is generating three types of signal files, these results also showed that, unsurprisingly, EpiLaP is sensitive to the signal type. We also wanted to use this preliminary version to help settle the metadata reinterpretation discrepancies from the GEO datasets. We therefore downloaded from ChIP-Atlas ~2k reprocessed samples related to the six core histone modifications, as well as their corresponding raw data from GEO that we reprocessed using the IHEC harmonization pipeline. Using this strategy, we identified 739 samples (35% of 2,101 total samples) with missing target information or discrepancies between ChIP-Atlas and NGS-QC. From these, 61% presented a confident prediction (>0.9). For samples with only one database interpretation (241 samples, 12%), 38% were mislabeled. Regarding the 1,121 consensus samples between databases (53%), we observed that 2% were mislabeled (wrong author's submitted metadata target protein).

Our next step is to train the EpiLaP v1 using this time the harmonized data from EpiAtlas as well as the three signal types to obtain a much robust model to validate the ~15k core histone modification samples reprocessed by ChIP-Atlas. Considering that the vast majority of GEO datasets seem to be correctly annotated, the natural follow-up will be to train a new version of EpiLaP greatly expanding on the number of predicted targets by using the ~60k ChIP-Seq reprocessed datasets.

SOX9 REPROGRAMS ENDOTHELIAL CELLS BY ACTING AS A DYNAMIC PIONEER TRANSCRIPTION FACTOR.

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During embryogenesis, the transcription factor SOX9 directs multiple developmental processes. SOX9 is activated upon endothelial-to-mesenchymal transition (EndMT), but its role in regulating this process is not clear.

Some developmental transcriptions factors have the remarkable ability to reprogram one cell type into another by engaging genes that are developmentally silent and in closed chromatin, thus acting as pioneer factors to initiate transcriptional events. In the present work, we have used human umbilical vein endothelial cells (HUVECs) to study cell reprogramming initiated by SOX9 and its role in restructuring the chromatin landscape. Transcriptome sequencing revealed that ectopic expression of SOX9 in endothelial cells is sufficient to induce expression of mesenchymal marker genes. In addition, the cells acquired increased invasive properties and a mesenchymal morphology. We used the chromatin profiling methods CUT&RUN and CUT&Tag to map SOX9 binding and histone marks (H3K4me1, H3K27ac, and H3K27me3). In addition, we mapped chromatin accessibility by ATAC-seq. Altogether, we found that at a subset of regions that are silent in unstimulated HUVECs, SOX9 occupancy increases chromatin accessibility and enrichment of active histone marks to induce gene expression, demonstrating SOX9's ability to act as a pioneer factor to reprogram cell fate. This pioneer function is motif encoded and occurs predominantly in distal regulatory regions. Our data also suggests widespread SOX9 chromatin scanning in silent chromatin, which does not result in chromatin opening. Furthermore, we show that at most pioneering sites,

SOX9 binding is highly dynamic, but nevertheless causes stable changes in the chromatin landscape and cell fate.

Our study highlights the crucial developmental role of SOX9 and provides new insight into key molecular functions of SOX9 and mechanisms of EndMT.

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. Intra-tumour heterogeneity of TNBC poses an unmet challenge for treatment, 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. Hence, we hypothesize that TNBC develops intra-tumour metabolic heterogeneity through the dynamic reprogramming of epigenetic landscapes in response to oxygen and nutrient availabilities within the tumour. Using multiplex immunofluorescence staining followed by spatial transcriptomics analysis of TNBC patient-derived xenografts (PDXs) and matched patient samples, we have identified two cancer cell populations within TNBC, respectively characterized by increased glucose or nitrogen metabolism. These two zones exhibit mutually exclusive spatial localization patterns and different distances to tumour vasculatures in multiple TNBC PDXs and matched patient samples. The trimethylation of lysine 27 on histone 3 (H3K27me3), which regulates chromatin accessibility and gene expression during development and differentiation, shows different levels in these two tumour zones, with lower H3K27me3 levels in highly proliferating, nitrogen metabolic zones. Accordingly, we show that inhibition of Enhancer of Zeste Homologue 2, the methyltransferase responsible for H3K27me3, results in increased amino acid signatures including nitrogen metabolism in TNBC models, suggesting a spatiotemporal H3K27me3-mediated regulation of nitrogen metabolism in TNBC. This project will decipher the mechanism of H3K27me3-mediated regulation of metabolic profiles within distinct tumour zones and will investigate how this epigenetic reprogramming modulates intra-tumour metabolic heterogeneity in TNBC.

HISTONE H2AK119 MONOUBIQUITINYLATION AFFECTS SKELETAL MUSCLE DIFFERENTIATION

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Skeletal muscle stem cells (commonly known as satellite cells) are essential 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 nuclei to regenerate the damaged muscle fibers. The activated myoblasts continue to expand their population, until ques which direct the cells to self-renew back into the stem cell population or differentiate into the muscle fibers occur. To govern the changes to these states, precise epigenetic regulation is required to ensure that the changes between cell states happen at the appropriate times and to ensure the correct number of cells self-renew or differentiate. Many different epigenetic factors 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 H3K27me3 and H2AK119ub1 catalysed by the Prc2 and Prc1 complexes respectively. The Prc1 complex also has major roles in chromatin compaction through the polymerization of Prc1 complexes together, which drives phase separation and strong repression of genes.

In the context of skeletal muscle, the Polycomb proteins regulate some aspects of the changes between these states. Roles of the Prc2 complex in preventing the expression of late myogenesis genes has been previously discovered, however insufficient studies have been performed to determine the roles of the Prc1 complex within myogenesis as well as to the precise role of this complex in the sequential addition of the Polycomb histone marks. Our work seeks to resolve the question as to what the role of the Prc1 complex is during myogenesis, and if it is necessary for skeletal muscle regeneration. Using enzymatic inhibitors to the Prc1 complex, we are analysing the role of the Prc1 complex in regulating the process of the myoblasts differentiating *in vitro*. Preliminary 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 find the role of the remainder of the complex. For this 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.

DYNAMIC REGULATION OF THE ATAD2B BROMODOMAIN ACTIVITY BY THE EPIGENETIC LANDSCAPE

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Bromodomains are protein domains that selectively recognize acetylated lysine residues. Different bromodomains exhibit a preference for specific patterns of post-translational modifications (PTMs) on core and variant histone proteins. In this study, we examined the ligand specificity the ATAD2B bromodomain and compared it to its closely related paralog in ATAD2. We show that the ATAD2B bromodomain recognizes mono- and di-acetyllysinemodifications on histones H4 and H2A. A structure-function approach using NMR titration experiments and site-directed mutagenesis coupled to ligand binding assays identified key residues in the acetyllysine binding pocket that dictate the molecular recognition process. We further characterized an alternative splice variant of ATAD2B that results in a BRD loss of function. Our results outline the structural and functional features of the ATAD2B bromodomain and identify a novel mechanism regulating the interaction of the ATAD2B protein with chromatin.

DNA METHYLATION PATTERNS OF THE PSYCHOSIS SPECTRUM IN INDIVIDUALS WITH 22Q11.2 DELETION SYNDROME

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22q11.2 deletion syndrome (22qDS) is a genetic syndrome caused by a chromosome 22q11.2 microdeletion, with a variable presentation that often includes congenital heart abnormalities, immune system deficiencies, distinctive facial features, developmental delays and psychiatric illnesses. Notably, 22qDS has the highest known penetrance for psychosis spectrum disorders (PSD), with ~25% of adults with 22qDS also developing a PSD such as schizophrenia. Recently, a DNA methylation signature of 22qDS was published, demonstrating a genome-wide differential methylation pattern specific to individuals with 22qDS, as compared to healthy controls. This is likely due, in part, to the haploinsufficiency of genes in this region which act in chromatin remodelling. Current work in DNA methylation signatures is becoming ever more sophisticated with growing evidence of, 1) unique signatures for multiple disorders mapping to a single gene, 2) signatures specific to disorder endophenotypes and 3) overlapping signatures across genes encoding subunits of a protein complex. Here, we present a new DNA methylation signature of 22qDS and evidence of differential DNA methylation patterns between individuals with and without PSD within a 22qDS cohort.

DNA extracted from blood and saliva was run on the Illumina Infinium HumanMethylation EPIC array. We applied a linear regression to array data and identified differentially methylated sites that met genome-wide significance (FDR p-value<0.05); these sites were then input into a machine learning algorithm to generate a predictive model of the presence of PSD in individuals with 22qDS. The methylation patterns specific to individuals with PSD were quantifiable in both blood and saliva, despite the genome-wide differences in DNA methylation between these tissues. These DNAm data provide insights into the genetic dysregulation associated with PSD in individuals with 22qDS. In future, we will determine whether these methylation patterns arise prior to presentation of PSD to allow for faster and more personalized medical interventions for individuals at higher risk of PSD.

MORE POWERFUL METHOD FOR DETECTING INFLUENCES ON BISULFITE SEQUENCING MEASURES OF DNA METHYLATION

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Recently graduated PhD student Kaiqiong Zhao developed methods for estimating the effects of multiple covariates on DNA methylation data obtained from targeted bisulfite sequencing. We developed a novel hierarchical varying coefficient regression method called SmOoth ModeliNg of BisUlfite Sequencing (SOMNiBUS - now a package on Bioconductor), which allows covariate effects to vary smoothly along genomic positions.

A specialized Expectation-Maximization algorithm allows for measurement errors in the methylated counts) and leads to both regional measures of association and pointwise tests and confidence intervals. SOMNiBUS allows for extraparametric variation by a hierarchical quasi-binomial varying coefficient mixed model that includes both multiplicative and additive dispersion and thereby captures the observed realistic dispersion trends. Power is substantially improved over competing methods. We have used this approach to analyze the differences in methylation patterns between ACPA+ and ACPA- individuals sampled from CARTaGENE.

GENOME GRAPHS DETECT HUMAN POLYMORPHISMS INACTIVE 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.

FUNCTIONAL CHARACTERIZATION OF THE MEST GDMR

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The proximal end of mouse Chr6 contains an imprinted domain comprised of the paternally expressed gene *Mest*, and the maternally expressed genes *Copg2* and *Klf14*. The *Mest* locus contains the only gametic DMR in the region, which overlaps the *Mest* promoter and exon 1. This domain shares syntenic homology with human Chr 7q32. Loss of MEST function may therefore contribute to the mUPD7 phenotype associated with Silver-Russell syndrome and characterized by growth retardation in humans. Although *Mest* overexpression has been reported to be associated with increased placental triglycerides in *Dnmt1o* KO placenta, we report no differences between genotypes in our single gene KO model. Here, we show that the *Mest* gDMR may play a wider role in gene regulation than previously thought.

We hypothesize the *Mest* gDMR may act as an Imprinting Control Center, regulating both *Mest* and *Klf14*. *Klf14* is an imprinted transcription factor implicated in metabolism, and is located almost 200kb from the *Mest* gDMR. Previous work showed that *Klf14* expression is lost in embryos lacking maternally inherited DNAme, genome wide. So, paradoxically, oocyte-derived DNAme is required for *Klf14* expression. Furthermore, CTCF has also been shown to bind to the unmethylated paternal allele of the *Mest* gDMR only, possibly establishing differential allelic subdomains/TADs which in turn regulate imprinted gene expression by restricting promoter-enhancer interaction.

To study regulation of imprinting at the *Mest* domain, we have generated *Mest* gDMR deletions in reciprocal F1 mouse embryonic stem cells by CRISPR-Cas9 mutagenesis. We have developed an *in vitro* endothelial model to study loss of imprinting, and show that *Klf14* imprinting is indeed regulated by the *Mest* gDMR. To identify promoter- enhancer contacts, and allelic subdomains, we have performed allele specific genome- wide Chromosome Conformation Capture (4C-seq). Our work provides further insight on the function of the *Mest*

imprinting domain, as well as on the broader mechanisms behind gene regulation.

SEX-SPECIFIC DNA METHYLATION IN PLACENTAL CELLS

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Placental sex, which is the same as the biological sex of the fetus, may influence structural and functional aspects of the placenta and as a consequence 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. Distinctive DNAm profiles between male (XY) and female (XX) placentae have been observed. DNAm also differs between different placental cell types. DNAm in bulk placental tissue reflects its cell type composition. However, characterization of human placental DNAm based on the interaction of both placental sex and cell type is poorly understood. This study investigates the sex-specific DNAm in placental cells and placental chorionic villi. We measured DNAm using the Illumina EPIC DNA methylation array for 68 male and 83 female term placentae in five cell populations: endothelial, stromal, Hofbauer, trophoblasts, syncytiotrophoblasts and whole chorionic villi. Using linear modelling with statistical cutoffs of $|\beta\text{ -value}| > 0.05$ and FDR < 0.1, we identified 151 and 15 autosomal sex-influenced CpGs in endothelial cells and chorionic villi respectively, but few/none sex differences were identified in other cell types. These sex-influenced CpGs shared partial overlap (24 endothelial and 7 chorionic villi sex-specific CpGs) with the sex-differentially methylated CpGs of chorionic villi from Inkster et al. (2021). To further investigate the findings in placental endothelial cells, we calculated the Pearson correlation coefficient between the sex-specific CpGs in endothelial cells with chorionic villi, as well as with human umbilical cord endothelial cells (HUVEC), umbilical cord white blood cells (UC-WBC) and chorionic villi from Herzog et al. (2017, Placenta 58:122-132). The DNAm data from Herzog et al. (2017) were derived from tissues extracted after delivery of the newborn and measured by the Illumina 450K methylation array. Chorionic villi (EPIC) and HUVECs showed a high correlation ($r = 0.64$) equal to the correlation between chorionic villi (450K) and endothelial cells ($r = 0.64$). However, endothelial cells and HUVECs presented a lower correlation ($r = 0.41$), while HUVEC and UC-WBC showed the highest correlation ($r = 0.96$). GO term enrichment analysis for endothelial cells did not reach statistical significance, possibly due to the small sample size. Our study provides the characterization of sex differences in autosomal DNAm in isolated placental cell types. The characteristics of DNAm considering both sex and cell type in the placenta will help identify sex differences associated with placental complications and pregnancy outcomes.

VARIANT-DEPENDENT CHROMATIN STATES IN THE MAMMARY SYSTEM

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Genome-wide association studies (GWASs) have highlighted how genetic variants can be linked to complex diseases. In some cases, GWAS variants contribute to downstream phenotypes through mutations within the coding region of involved proteins that directly affect function. However, an overall view of GWASs shows that nearly 90% of phenotype-associated single-nucleotide polymorphisms (SNPs) lie within non-coding regions. Recent literature has begun to track causal links between genetic variants inside and outside of coding regions, and transcription-modulating epigenetic states in order to clarify how changes in epigenetic features orchestrate the flow from genetic variation to phenotypic outcome. This area of research has the potential to identify both epigenetic markers and therapeutic targets of disease-linked genetic variants. However, these studies rely either on cell lines, whose epigenetic state is modulated by their method of preparation, or non-purified tissues with heterogenous cell types. Given the epigenetic specificity of cells depending on environmental medium and differentiation state, as well as known divergences in the epigenome between cell lines and primary cells, there is little knowledge available as to how genetic variants can have epigenetic consequences unique to primary cell types in a single individual. Previous work has characterized the unique epigenetic features of four purified primary mammary cell types: basal cells, luminal cells, luminal progenitors, and stromal cells. This data provides a promising starting point to investigate how variants can drive epigenetic features across individuals, and how this can be modulated in each cell type.

Here we profile the existence and cell-type specificity of variant-associated histone marks in these 4 cell types among 8 individuals. This study has the potential to illustrate how the impact of variants linked with epigenetic state changes are modulated by existing differences in transcription factor or transcriptional landscapes inherent to different cell types of the breast system. This may illuminate how variant-driven phenotypes and diseases linked to changes in epigenomic state could manifest differently depending on cell type.

IN VIVO CRISPR SCREENS IDENTIFIED DUAL FUNCTION OF MEN1-MLL1 IN REGULATING TUMOR-MICROENVIRONMENT INTERACTIONS

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BACKGROUND: CRISPR functional genomic screens have been widely adopted to identify essential genes and potential drug targets in cell line models. However, it is well known that

cell line models studied in vitro do not fully capture the biology in patient tumors due to the lack of the tumor microenvironment. The primary objective of this study was to perform functional genomic screens in *in vivo* models to identify clinically relevant epigenetic vulnerabilities.

METHODS: We designed an EpiDrug sgRNA library that target 357 epigenetic regulators in human and applied this targeted sgRNA library for essentiality screen in 2D cell culture and 3D xenograft models.

RESULTS: We identified MEN1 as the top hit that confers differential essentialities between in vitro and in vivo models. Knockout of MEN1 has no impact on cell proliferation in 2D cell culture but profoundly promotes tumor growth in human lung and colon cancer cell line derived xenograft models. In syngeneic colon cancer model CT26, knockout of Men1 resulted in faster tumor growth in immune deficient mice, but reduced tumor growth in immune competent mice. Mechanistically, knockout of MEN1 activates a group of genes involved in cytokine-cytokine receptor interaction, through redistribution of MLL1 chromatin interaction to repeat regions and activation of the viral mimicry response. Single-cell and CyTOFanalysis revealed that activation of the cytokines induces tumor promoting neutrophil and tumor suppressing CD8+ T cell infiltration in immune deficient and immune competent mice, respectively. MEN1 inhibition dramatically reduced CT26 tumor growth and demonstrated strong synergy with anti-PD-L1 in immune competent mice.

CONCLUSION: Our study demonstrated the utility of in vivo CRISPR screen in identifying therapeutic targets that modulate tumor-microenvironment interactions and identified MEN1 as a promising therapeutic target alone or in combination with immunotherapy.

KDM5 LYSINE DEMETHYLASE FAMILY SUBSTRATE PREFERENCE AND IDENTIFICATION OF POTENTIAL METHYLLYSINE-CONTAINING PROTEIN SUBSTRATES

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A major regulatory influence over cell biology is the dynamic post translational modification (PTM) of proteins. Specifically, lysine methylation/demethylation within histone proteins has been documented to be an important mechanism of regulating gene expression. The KDM5/JARID1 sub-family are 2-oxoglutarate and Fe(II)-dependent lysine-specific histone demethylases that are characterized as such by their Jumonji catalytic domains. This enzyme family has been well-established to facilitate the removal of tri- and dimethyl modifications from lysine 4 of histone H3 (i.e., H3-K4me2/3), a mark associated with active gene expression. As a result, studies to date have predominately revolved around KDM5's influence on disease progression through their ability to regulate gene expression via H3-K4 demethylation. Recently, there is growing evidence that KDM5 enzymes may influence disease beyond histone demethylation of the H3-K4 site. This insight has made it critical to further investigate KDM5 demethylation activity towards non-histone proteins. In efforts to help identify potential non-histone substrates for the KDM5 family, we developed a library of 180 permutated peptide substrates, with sequences that are systematically altered from the WT H3-K4me3 sequence. From this library, we characterized recombinant $KDM5A_{1-800}$, $KDM5B_{1-821}$, $KDM5C_{1-765}$, $KDM5D_{1-775}$ substrate preference and subsequently, developed recognition motifs for each KDM5 demethylase. The recognition motifs developed were used to predict potential substrates for KDM5A/B/C/D. Demethylation activity was then profiled to generate a priority list of high-ranking and medium/low-ranking substrates for further *in vitro* validation for each of KDM5A/B/C/D. Through this approach, we identified 48 high-ranking substrates in which KDM5 demethylases displayed significant *in vitro* activity towards. Herein, we look to validate and explore the cellular significance of the KDM5 family mediated demethylation of several of these candidate proteins. One of which is MTA1-K532, a methylation site with known functional significance.

ZIC2/3/5 FACILITATE EXIT FROM NAIVE PLURIPOTENCY AND STABILIZES A PRIMED STATE

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Embryonic development entails co-ordinated dynamic changes in regulatory circuits driven by strict epigenetic mechanisms. One developmental window in which this phenomenon is significant is during peri-implantation, when the naïve epiblast undergoes implantation and forms the primed epiblast, which then gives rise to the germ layers that form every cell of the human organism. Interestingly, we can model this developmental window and the underlying regulome using pluripotent stem cells that are derived from the human embryo and cultured in defined media conditions. ATAC- seq analysis of primed compared to naïve pluripotent stem cells (PSCs), shows a marked increase in open chromatin regions containing DNA motifs corresponding to ZIC2 – a transcription factor upregulated in primed PSCs. We have conducted ChIP-seq of ZIC2 in primed PSCs and shown that ZIC2-bound regions in primed PSCs largely acquire characteristics associated with active enhancers and bivalent promoters. ZIC2 deletion, using CRISPR-Cas9 results in reduced accessibility over regions generally bound by ZIC2, however, despite strong correlation between bivalency and ZIC2 occupancy, there is no striking loss of H3K27me3 over ZIC2-bound regions in ZIC2-null primed hESCs. This is in contrast to a recent study in mESCs, in which loss of ZIC2 results in loss of facultative heterochromatin. In addition, ZIC2 occupies many primed-specific putative enhancers either to maintain expression of pluripotency genes

like POU5F1 or express primed-specific genes like DNMT3B. Orthogonally, ectopic expression of ZIC2 in naïve hESCs results in increased accessibility over regions that go on to be subsequently occupied by ZIC2 – many of which are specifically open in primed hESCs and not naïve hESCs. This results in a shift from the naïve transcriptome to a more primed hESC-like transcriptome, and an upregulation of many putative downstream targets of ZIC2 like FOXO4. Furthermore, we observe reduced viability of primed hESCs harboring deletion of both ZIC2 and ZIC3 compared to primed hESCs lacking either ZIC2 or ZIC3, and increased spontaneous differentiation in primed hESCs deficient of ZIC2 and ZIC5. This would suggest that ZIC2, in addition to having unique targets, also have targets that are redundant with ZIC3 and ZIC5; and that, ZIC2/3/5 work in tandem to maintain pluripotency and self renewal capacity of primed hESCs. Indeed, human ZIC2 and human ZIC3 have strong homology in amino acid sequences and a comparison of ChIP-seq profiles of ZIC2 and ZIC3 in primed hESCsshow that regions bound by ZIC2 are also likely to be bound by ZIC3. These results suggest a dual role for ZIC2 in maintaining pluripotency in primed hESCs and facilitating exit from naïve pluripotency, via opening or maintaining an open state of enhancers. Overall, through this and future studies, we hope to define the role of ZIC2 in faithful progression from naïve to a primed pluripotency state.

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 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 3558 histone mark ChIP-seq samples across tissues from the International Human Epigenome Consortium (IHEC) 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 find some significant differences in TE enrichment between cell types and that TE enrichment can be cell type specific in certain contexts. We also present a generalised profile of the relationship between TE enrichment which reveals a few TE families (Alu, MIR, L2) as potential genome regulators. These results further support existing research while highlighting novel associations and providing a comprehensive profile of TEs across cell types and histone marks.

WHO'S AFRAID OF THE X? INCORPORATING SEX CHROMOSOMES INTO THE ANALYSIS OF ILLUMINA DNA METHYLATION MICROARRAY DATA

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Background: Biological sex differences are observed in many human phenotypes, and sex-based analyses in health and disease are gaining popularity. It studies of biological sex it is critical to consider the sex chromosomes, but while the X and Y are interrogated in genome-wide assays such as DNA methylation (DNAm) or sequencing experiments, XY data are rarely investigated due to analytical complexities associated with dosage disparity and X-chromosome inactivation. Development of analytical methods for XY data is critical to further the study of sex as a biological variable. We hypothesized that XY DNAm microarray data require processing and analysis techniques unique from those employed with autosomal data.

Methods: Using an Illumina 450K dataset (702 placentas) we systematically assessed the effect of all processing and analysis steps on XY data.

Results: Several processing steps required amendment for sex-linked data. First, XY DNAm beta values are directly associated with XY copy number, so genetic sex was carefully evaluated by assessment of total fluorescence from XY probes with follow-up for karyotypically abnormal or contaminated samples. Copy number assessment was performed using DNAm data with the conumee package, with which we discovered one sample with a putative 45X/46XX karyotype. Contamination of male samples with female DNA, common in prenatal tissues due to maternal contamination, shifts X chromosome DNAm beta values in male samples; several samples of both sexes from public datasets were identified as highly contaminated and excluded. In terms of probe filtering, Y chromosome detection P values should be evaluated only in male samples, as the majority (87.5%) of Y probes fail when considering both sexes, compared to 0.7% when sex-stratified. Sex stratification did not affect other filtering steps, though if removing non-variable probes, stratification is recommended to avoid underestimation of non-variable Y probes (0 in both sexes, versus 37 or 8.8% in males only). When excluding probes overlapping single nucleotide polymorphisms (SNPs), 2/4 common databases do not sufficiently index XY SNPs; the

database used must be selected carefully when working with XY data. The same is true of non-specific probes: common resources have extremely variable coverage of XY probes and should be chosen carefully. Using technical replicates, we determined that most common normalization methods preserve biology-related XY DNAm patterns, and that batch correction is suitable for XY data. After processing, we obtained 9560 X and 170 Y CpGs suitable for analysis. We also extended our findings to the more recent EPIC array (66 placentas) with similar results.

Conclusions: XY DNAm microarray data can be utilized with key modifications to standard preprocessing and analyses steps. Additionally, we propose a set of general recommendations for analysis of XY data based on study design to appropriately consider X-chromosome inactivation.

BEYOND THE NUCLEUS: ELUCIDATING THE INTERACTOME OF RETINOBLASTOMA BINDING PROTEIN 5

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Retinoblastoma Binding Protein 5 (RbBP5) is a member of the WRAD complex, a multi-subunit complex of proteins that associates with and elevates the catalytic activity of the SET1 family of histone methyltransferases. Interestingly, recent findings showing that SET1 methylates cytoplasmic proteins highlighted non-nuclear activity of the complex. Moreover, preliminary data from our lab has shown that RbBP5 localizes outside of the nucleus and can interact with cytoplasmic proteins. These observations led to the hypothesis that RbBP5 is involved in other cellular processes, beyond epigenetic signaling. To examine this hypothesis, the protein complexes scaffolded by the cytoplasmic form of RbBP5 were immunoprecipitated and identified by mass spectrometry. This analysis revealed 25 novel cytoplasmic binding partners for RbBP5. Comparison of the interactions using full-length RbBP5 and a truncated construct indicated the potential binding site – namely, the N-terminal β-propeller domain or the C-terminal wire – for each partner. Interestingly, the newly identified interactors are associated with a wide variety of cellular events, including cell adhesion, immunity, mRNA splicing, and more. Further investigations will allow to decipher the specific roles of RbBP5 within these biological processes, such as scaffolding the different protein complexes, mediating target binding, or other. In addition, WDR5 (another WRAD complex subunit) was found to interact with cytoplasmic RbBP5, pointing to the presence of the methyltransferase complex outside of the nucleus. This finding also suggests that the newly identified RbBP5 interactors may themselves be targets for methylation by the SET1 enzymes. Additional work will aim to probe the assembly of the SET1 methyltransferase complex within the cytoplasm, and decipher the potential methylation profiles of the newly identified RbBP5 interactors. Overall, this work aims to provide critical foundational knowledge pertaining to RbBP5 biology and functions, beyond the context of histone lysine methylation.

STRUCTURE BASED SCREENING OF POTENT AND SELECTIVE SMALL MOLECULE INHIBITORS TARGETING ASH2L-RBBP5 COMPLEX

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Approximately ~22,000 Canadians are living with or are in remission from leukemia (llscanada.org). The Trithorax family of *Hox* gene regulatory proteins called the Myeloid Lymphoma Leukemia (MLL) protein is upregulated and mutated in acute or chronic forms of leukemias. In specific blood cancers, the ability of MLL to methylate histone H3 on Lys-4 is essential in the oncogenic process. Mediated by its SET domain, MLL1 methyltransferase activity strictly relies on its interactions with four proteins, including WDR5, Ash2L, RbBP5, and DPY30. Ash2L is essential for embryonic development and global H3K4me3. Through its interaction with Ras, Ash2L also acts as a potent oncogene. Ash2L employs two distinct regions to interact with RbBP5 and DPY30. The SPRY domain of Ash2L binds to RbBP5, and its C-terminus binds to DPY30. Using mutational analysis and genetic approaches, we have demonstrated that disruption of each complex leads to a loss of H3K4me3 and target gene expression.

We propose using a combination of *in silico*, *in vivo*, and *in vitro* approaches to test the impact of small molecules or biologics blocking the formation of Ash2L-RbBP5 complex and their effects on the proliferation of leukemic cell lines. Fluorescence polarization competition assays have been established in a high-throughput format for screening small molecules to identify new inhibitors that disrupt Ash2L-RbBP5 interaction. We applied peptide displacement assay to screen RbBP5 against a targeted library of compounds and identified a potent inhibitor that targets the Ash2L-RbBP5 complex.

We are in the process of biochemically and biologically validating these molecules. Ultimately, our studies will identify novel molecules targeting this complex for treating patients who have leukemia and other MLL linked diseases.

SINGLE-CELL MULTIOMICS IDENTIFY THE TRAJECTORY OF POPULATIONS DRIVING LOCALLY ADVANCED PROSTATE CANCER

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Prostate cancer (PC) is one of the most common and most heterogeneous cancers in men. One in six patients present with locally advanced disease and have tumour dissemination in their pelvic lymph nodes upon diagnosis. The clinical course for these patients is highly variable with a worse prognosis. Large scale sequencing-based studies have identified the contribution of genetic and non-genetic aberrations within the chromatin to prostate carcinogenesis. However, the distinct cell states underlying individual PCa tumours are still incompletely understood. Phenotypic diversity and specific cell state result from a complex system of chromatin, DNA-binding proteins such as transcription factors, and the transcriptome. To identify the underlying pathways driving cancer aggressivity, we profiled the chromatin accessibility and transcriptome at a single-cell resolution. We present data from a single patient across six tumour sites and its five matched lymph node biopsies, with a total of 104240 high-quality cells. We demonstrate significant heterogeneity in the transcriptional and chromatin landscapes of malignant cells between multiple biopsy sites. Using chromatin accessibility data and lineage tracing, we showcase dynamic modules of *cis*-regulatory elements that contribute to aggressive phenotypes. 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 aggressive cell states, which provide new cancer-specific vulnerabilities that advance the identification of therapeutic targets in the era of precision medicine.

CHARACTERIZATION OF MIXED LINEAGE LEUKEMIA PARTIAL TANDEM DUPLICATION IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is one of the most aggressive blood cancers. This disease is characterized by abnormal and rapid proliferation of hematopoietic cells of the myeloid lineage. The histone-3-Lysine-4-methyltransferase gene *Mixed Lineage Leukemia 1* (MLL), is essential for embryonic hematopoietic stem cells development maintenance and crucial in cell fate decision during hematopoiesis. When first produced in the cytoplasm, MLL is cleaved into two subunits to form the MLL N & C-terminus regions that re-associate upon entry in the nucleus. MLL also interacts with the WRAD complex, including WDR5, RBbp5 Ash2L and DPY-30 proteins, which is essential for its catalytic activity. AML patients exhibit frequent mutations of MLL, the most common of which involve fusion of the MLL C-terminal part with various partners (e.g. AF4). In addition to MLL fusions, the MLL gene can also be subjected to Partial Tandem Duplication (PTD) mutations that lead to duplication of a portion of the MLL-terminal domain that contains AT hooks and CXXC domains. While the mechanism of MLL fusions-mediated leukemogenesis has been established, it remains unknown how the MLL-PTD mutant that preserves an intact enzymatic activity leads to leukemic transformation.

To better understand the role of MLL-PTD in leukemogenesis, we used a combination of cell biology and proteomic approaches in leukemic cell lines and patient-derived xenograft models (PDX) of AML that express MLL-PTD. Surprisingly, we discovered that MLL-PTD loses interaction with a number of cofactors known to interact with its wild type counterpart. On the other hand, MLL-PTD acquires novel interacting partners, which are likely to profoundly influence its gene regulatory activity. Also interestingly, our mass spectrometry results indicate a perturbed stoichiometry between the C-terminal and N-terminal portions of MLL-PTD, suggesting deregulated structure and function.

Taken together, our results provide important new insights into our understanding of the mechanism of MLL-PTD mediated leukemia.

INCREASED COMPLEXITY IN THE TATA-BINDING PROTEIN FAMILY OF PROTEINS

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Highly conserved throughout evolution, the TATA-box binding protein (TBP) is an essential transcription factor for Pre-Initiation Ccomplex (PIC) nucleation in the three main eukaryote RNA Polymerases. However, as organisms evolve and control of gene expression becomes increasingly more complex, metazoans have evolved distinct TBP paralogs that have specialized functions to account for this complexity. For example, in oocyte, TBP is completely absent whereas TBP2 is highly expressed and functionally replaces the role of TBP in RNA Polymerase II (Pol II) transcription. However, it remains unclear how distinct or redundant these paralogs are in Pol II-mediated transcription. Here, we report that degradation of TBP via the minimal auxin-inducible degron (mAID) system in mouse embryonic stem cells (mESCs) has no global effect on Pol II transcription. Subsequent activation of repressed genes via retinoic acid differentiation is also not perturbed in the absence of TBP. Instead, we show that a metazoan-specific paralog of TBP (TRF2) is expressed in mESCs and that it binds to promoter regions of active or activated Pol II genes even in the absence of TBP. Taken together, our findings reveal that TRF2 has the potential to replace TBP in mESCs, and how the initiation mechanism for Pol II has evolved and diversified over time.

DISTINCT H3K27ME3 DISTRIBUTION IN HUMAN TROPHOBLAST AND EMBRYONIC STEM CELLS

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The first cell lineage specification in human embryogenesis occurs when the morula differentiates into a blastocyst, comprising inner cell mass and an outer layer. The inner cell mass differentiates into the hypoblast, which gives rise to extraembryonic tissues such as yolk-sac, and the epiblast, which gives rise to the fetus. The outer layer becomes the trophoblast, which eventually forms placenta. The placenta is an essential organ for fetal life, creating a suitable environment and providing nutrients to the developing embryo. The epigenome of the placenta, the DNA methylation and histone modifications which regulate gene transcription, differs dramatically from that of embryonic tissues. Understanding the difference in heterochromatin establishment of trophoblast and embryonic tissues can contribute to explaining their early developmental separation and distinct DNA methylation profiles. To compare the genome-wide distribution of repressive histone modifications in early lineages, we performed ChIP-seq of two repressive histone marks H3K27me3 and H2AK119ub in human trophoblast stem cells (hTSCs), naïve human embryonic stem cells (hESCs), and primed hESCs, which correspond to mature trophoblast, mature epiblast, and early embryo respectively. Our results showed that both H3K27me3 and H2AK119ub vary in abundance and distribution. Among three cell types, primed hESCs showed highest enrichment for both H3K27me3 and H2AK119ub that are localized to TSS. Large domains of relatively lower enrichment for H3K27me3 throughout the genome were observed in hTSCs. These differences in repressive histone distribution suggest that placenta may have specific mechanism of epigenetic regulation, different from that of embryonic tissues.

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. Importantly, analyses of clinical specimens revealed 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 revealed an increased RNA polymerase II (Pol II) promoter-proximal pausing at AR-dependent genes following MYC overexpression without an accompanying deactivation of AR-bound enhancers. 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.

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PATHOGENIC DNMT3A MUTATIONS LEADS TO ALTERED DNA METHYLATION PROFILES IN INDUCED-PLURIPOTENT STEM CELLS AND NEURAL PROGENITORS

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Heterozygous mutations identified in the functional domains of DNMT3A (DNA methyltransferase 3A) interfere with normal brain development. DNMT3A is an enzyme responsible for establishing DNA methylation implicated in gene regulation, and vital for development and cellular identity. Since no functional studies have been performed on heterozygous DNMT3A mutations associated with abnormal brain development in human, we do not know how they cause alterations in DNA methylation, or how this affects the specification of DNA neuronal cell lineage and relevant differentiation programs.

With the collaboration of clinical geneticists, we have identified 2 patients, carrying a single mutation in the functional

methyltransferase domain of DNMT3A. Using cells from those patients, we derived induced-pluripotent stem cells (IPSC) that we then differentiated into neural progenitors (NPC) to specify the deleterious impacts of functional DNMT3A mutations on brain cell development. Using Methyl-Seq, we established DNA methylation profiles of both control and DNMT3A-mutated IPSC and NPC to identify differentially methylated regions (DMRs; ±>20 % of methylation difference). We show that the *DNMT3A* mutation causes induced 66 523 DMRs (methylation gain n=42 862, methylation loss n= 23 661) in the IPSC, and 165 844 DMRs (methylation gain n=91 699, methylation loss n=74 145) in NPC. In IPSC's, altered methylation was mainly observed in CpG dens regions (CpG islands) and in promoters, whereas in NPC's they were found in CpG islands, enhancers, and gene bodies. Dysregulated DNA methylation profiles were associated with genes implicated in development and neurodevelopment.

Our results show that pathogenic DNMT3A mutations affect the normal programming of pluripotent stem cells and interferes with the establishment of proper DNA methylation profiles during the initial steps of neural lineage specification. These results suggest that, as pluripotent stem cell undergo cellular differentiation and lineage commitment, the negative impact of pathogenic DNMT3A mutations on the distribution of DNA methylation marks intensifies, thus contributing to the abnormal developmental outcomes.

FIRST TRIMESTER PLASMATIC MICRORNA LEVELS PREDICT RISK OF DEVELOPING GESTATIONAL DIABETES MELLITUS

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Background: Gestational Diabetes Mellitus (GDM) is the most common pregnancy complication with a prevalence that can reach 25% depending on ethnicity and GDM diagnostic criteria applied. GDM has serious health consequences for both the mother and her child. GDM is currently diagnosed between the 24th and 28th weeks of pregnancy with an oral glucose tolerance test. Although the treatment of GDM contributes to prevent pregnancy and delivery-related complications, its effects on long term complications remain unclear. Earlier prediction of GDM could thus help improve follow-up and prevent longer term complications. MicroRNAs (miRNAs) are short single stranded RNA molecules (19-24 nucleotides) involved in post-transcriptional regulation through binding to their target messenger RNAs. They are secreted and stable in blood where they might well have endocrine functions.

Hypothesis: The miRNA profile of women developing GDM is dysregulated as early as the 1st trimester of pregnancy.

Objective: To identify plasma miRNAs measured in the 1st trimester of pregnancy predicting women at higher risk of developing GDM.

Methods: Using next generation sequencing, we have quantified miRNAs in 443 (n GDM=56) and 139 (n GDM=76) plasma samples collected at 9.6 and 11.9 weeks in Gen3G and 3D prospective birth-cohorts. miRNAs associated with GDM were identified using the DESeq2 package ($p<0.05$). Those common between Gen3G and 3D cohorts were selected for further analyses. A stepwise logistic regression was applied on a subset of the Gen3G cohort (70%; training set) to select miRNAs independently associated with GDM. The pROC package was then applied to build receiver operating characteristic (ROC) curves (test set: 30% of the remaining samples in Gen3G) and to compute Youden's index to assess specificity and sensitivity of the test.

Results: We identify 73 miRNAs associated with GDM in Gen3G ($p<0.05$). Of these, 17 miRNAs were replicated in 3D cohort ($p<0.1$). A logistic regression model including only 3 miRNAs (retained after stepwise logistic regression in the training set) has an area under the curve (AUC) of 0.78 (0.62-0.94), a specificity of 0.85 and a sensitivity of 0.71 in the test set. This miRNA selection was replicated in the 3D cohort with an AUC of 0.67 (0.58-0.76), a specificity of 0.66 and a sensitivity of 0.63. In comparison, classic GDM risk factors (age, BMI, Hb1Ac and 1h post-GCT glycemia) has an AUC of 0.81 (0.70-0.92), a specificity of 0.67 and a sensitivity of 0.92. A model combining miRNAs and classic risk factors has an AUC of 0.90 (0.85-0.97) a specificity of 0.87 and a sensitivity of 0.86.

Conclusion: We have found 17 plasmatic miRNAs measured during the 1st trimester of pregnancy associated with GDM development on average 4 months later. Of these, three predict women at higher risk of developing GDM with very good specificity while addition of classic risk factors better sensitivity.

EARLY PREIMPLANTATION ALCOHOL EXPOSURE INDUCES SEX-SPECIFIC DNA METHYLATION DYSREGULATIONS IN LATE-GESTATION PLACENTAS

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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) profiles and function of the placenta 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 placenta.

Our objectives are to identify if early embryonic ethanol-exposure leads to DNA methylation errors in the placenta 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 placentas of ethanol-exposed and control (saline) embryos. We then established genome-wide quantitative DNA methylation profiles of placentas samples (controls; 3 males and 3 females, ethanol-exposed; 5 males and 5 females) by Methyl-Seq and performed 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 991 differentially methylated regions (DMRs; 100bp regions with >10% methylation differences) in male placentas and 1309 DMRs in female placentas, with only 21 regions commonly affected in both sexes. In all our analyses, we observed a majority of DMRs with increased methylation in ethanol-exposed placenta. Interestingly, gene ontology analysis of genic DMRs in male placentas are related to synaptic transmission, neuron development and morphogenesis, whereas in female genic DMRs gene ontology reveals implication in inflammation, cell morphogenesis and cytoskeletal organization.

Our results show that an early acute alcohol exposure generates long-lasting sex-specific DNA methylation perturbations in the developing placenta. Current analyses are being done to associate specific DNA methylation dysregulations in both the placenta and brain of ethanol-exposed embryos. Since there is no molecular diagnostic test for FASD, specific DNA methylation dysregulation in the placenta could be used as a potential biomarker for prenatal alcohol exposure.

CHARACTERIZATION OF HERITABLE EPIGENETIC DYSREGULATIONS IN PROMOTER REGIONS FOLLOWING A TEMPORARY LACK OF DNMT1 IN MOUSE EMBRYONIC STEM CELLS

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During early embryo development, a major epigenetic reprogramming wave erases and re-establishes DNA methylation (**DNAmet**) profiles across the genome. However, specific regions such as imprinting loci must escape this reprogramming wave and maintain their precise DNAmet profiles by constant DNMT1 (DNA methyltransferase 1) activity to ensure the proper development. Using a mouse embryonic stem (**mES**) cell model with inducible *Dnmt1* repression (*Dnmt1*$^{tet/tet}$), we previously showed that the temporary loss of *Dnmt1* triggers the permanent loss of DNAmet profiles on imprinted and imprinted-like regions, as well as on other regions across the genome. We still do not understand why particular genomic sequences are unable to re-establish their normal DNAmet profiles following *Dnmt1* re-expression, and how other epigenetic marks (e.g., histone modifications) are robustly altered.

Here we aim to investigate how a temporary lack of DNAmet maintenance remodels the chromatin landscape on regulatory regions (i.e., promoters) and how this is associated with altered gene expression. To do so, we collected mES$^{Dnmt1tet/tet}$ cells prior *Dnmt1* inactivation, after *Dnmt1* inactivation, and following complete reactivation of *Dnmt1* expression. We then performed ChIP-Seq for histone marks (H3K4me3, H3K4me1, H3K27me3 H3K27ac), RRBS for DNA methylation (RRBS) and RNA-Seq for gene expression. By defining a list of 18,166 promoters we categorized them in four categories (Active, Bivalent, Depleted and Repressed). The inactivation of *Dnm1* lead to an enrichment of the activating histone mark (H3K4me3) for active, bivalent and a subset of repressed promoters, as well as a lower enrichment of the repressive histone mark (H3K27me3) for bivalent and repressed promoters. The complete reactivation of *Dnmt1* was able to rescue most altered histone mark profiles across promoter categories, as well as gene expression. However, across our promoter categories, we observe numerous histone marks that are un-enable to comeback at their original level without necessarily causing altered gene expression. Contrarily, epigenetic (i.e., DNA methylation, histone modifications) dysregulations found on promoters of imprinted and imprinted-like (i.e., *Xlr family*) genes as well as on imprinting control regions of imprinted genes (e.g., *H19*, *Meg3*) were linked to an altered gene expression.

Our results show that a temporary lack of *Dnmt1* has a greater impact on the conservation of histone marks than it has on the maintenance of DNAmet profiles on promoter regions in mouse embryonic stem cells.

DNA METHYLATION CUES IN NUCLEOSOME GEOMETRY, STABILITY, AND UNWRAPPING

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Cytosine methylation at the 5-carbon position is an essential DNA epigenetic mark in many eukaryotic organisms. Although countless structural and functional studies of cytosine methylation have been reported in both prokaryotes and eukaryotes, our understanding of how it influences the nucleosome assembly, structure, and dynamics remains obscure. Here we investigated the effects of cytosine methylation at CpG sites on nucleosome dynamics and stability. By applying long molecular dynamics simulations, we generated extensive atomic level conformational full nucleosome ensembles. Our results revealed that methylation induces pronounced changes in geometry for both linker and nucleosomal DNA, leading to a more curved, under-twisted DNA, shifting the population equilibrium of sugar-phosphate backbone geometry. These conformational changes are associated with a considerable enhancement of interactions between methylated DNA and the histone octamer, doubling the number of contacts at some key arginines. H2A and H3 tails play important roles in these interactions, especially for DNA methylated nucleosomes. This, in turn, prevents a spontaneous DNA unwrapping of 3-4 helical turns for the methylated nucleosome with truncated histone tails, otherwise observed in the unmethylated system on several microseconds time scale. We can conclude that 5-cytosine methylation might not only make available specific binding sites or occlude binding of regulatory proteins, but in addition, have a direct role in inhibiting the DNA unwrapping and regulating DNA accessibility. This additional mechanism could be employed by factors that can bind to nucleosomes.

NUCLEAR LOCALIZED TFPI AND THE DEVELOPMENT OF MULTIPLE DRUG RESISTANT CANCER

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Chemotherapy is an effective cancer treatment. Yet, a cancer can develop multiple drug resistance (MDR) to chemotherapeutics rendering them ineffective. Given enough time MDR occurs almost universally, and once developed applies in a non-specific manner. Because of its lack of specificity MDR renders many drugs, including those the patient has no pervious exposure to, ineffective, and severely limits treatment options.

The coagulation regulator tissue factor pathway inhibitor 1 (TFPI1) normally inhibits the tissue factor pathway thus preventing formation of blood clots. However, TFPI1 is highly elevated in MDR cancers where it's believed to mediate the development of drug resistance. Not only has increased TFPI1 abundance been observed in multiple different cancer types, but artificial overexpression of TFPI1 in cell lines has been shown to result in the development of an MDR phenotype. In MDR cancer cells TFPI1 does not localize to the cell surface as normal, but instead appears in the nucleus, and a putative nuclear localization sequence has been identified within the protein. We believe the accumulation of TFPI1 in the nucleus, mediated by the putative localization sequence, allows TFPI1 to promote MDR development.

The anaphase promoting complex (APC) is a nuclear E3 ubiquitin ligase and highly conserved regulator of cell cycle progression. Defects in APC function are associated with reduced longevity, and cancer. We believe the APC may also play a role in the promotion of MDR by TFPI1. Our observations suggest APC dysfunction exists in MDR cancers where APC substrates accumulate, as does TFPI1. We hypothesize that nuclear TFPI1 is an APC substrate, and that APC activation will result in TFPI1 degradation slowing MDR development. Our results have shown that activation of the APC reduces not only known APC substrate abundance, but also the abundance of TFPI1.

To summarize TFPI1 drives development of MDR cancer, for which we believe TFPI1's accumulation within the nucleus is important. Our future research will explore two aspects of TFPI1 driven MDR. First, we plan to express TFPI1 containing a mutation to this putative localization sequence to confirm the sequences' role in translocation to the nucleus, and the subsequent development of MDR. Second, we will track TFPI1 abundance in human MCF7 breast cancer cells undergoing selection for MDR along with APC activation and inhibition, so we can follow the effects of APC activity on resistance development. Both approaches have the potential to elucidate means to slow MDR development in cancer and extend chemotherapy effectiveness.

CELL-TYPE SPECIFIC TRANSCRIPTOMICS AND EPIGENETICS IN THE POST-MORTEM DORSOLATERAL PREFRONTAL CORTEX IN MDD

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Major depressive disorder (MDD), a serious and debilitating mental illness which affects over 260 million people worldwide, involves distinct neuronal, astrocytic, oligodendroglial, microglial, and endothelial contributions. Thus cell-type specific studies of the brain can inform us further about the relative contributions of these cell-types to the MDD phenotype. Moreover, there are sex differences in the clinical and molecular phenotype of MDD, providing a strong impetus to study both

males and females. Single-nucleus RNA-sequencing (snRNA-seq) allows us to investigate disease-associated transcriptomic changes within computationally defined cell-types or states in complex organs with heterogeneous cell-type composition, such as the human brain. We previously performed snRNA-seq in the post-mortem dorsolateral prefrontal cortex (dlPFC) in a cohort of male subjects who were either psychiatrically healthy (n=17) or were depressed and died by suicide (n=17). Our analyses revealed that a subtype of deep layer excitatory neurons and immature oligodendrocyte precursor cells showed the highest numbers of differentially expressed genes (DEGs). We further characterized the excitatory neuronal subtype by identifying genes which are highly expressed within it and confirming that this cellular subtype can be stably detected across independent snRNA-seq datasets. Next, we optimized a protocol for fluorescence assisted nuclei sorting (FANS) of this population from the post-mortem brain for future DNA methylation studies with a novel enzymatic methylation sequencing approach suited for low-input samples. Further, we expanded our snRNA-seq cohort to include depressed female subjects who died by suicide (n = 20) and matched controls (n = 18) and recapitulated the cell subtypes previously identified in the male snRNA-seq cohort. Future work will include meta-analysis of snRNA-seq results from the male and female cohorts, encompassing over 150000 cells, to identify consistent and distinct cell-type specific molecular changes across the sexes and investigation of epigenetic changes within isolated cell subtypes of interest.

INVESTIGATING ATAD2 FUNCTION - HOW THE BROMODOMAIN READS THE EPIGENETIC HISTONE CODE

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ATAD2 is a nuclear protein that is over-expressed in a multitude of cancers and is associated with poor patient outcomes. ATAD2 contains a bromodomain at its C-terminus, whose function is to “read” and interpret epigenetic modifications on histone tails, and specifically recognizes the acetylated lysine post-translational modifications. The bromodomain of ATAD2 is a structurally conserved motif consisting of a left-handed alpha helical bundle and was previously shown to recognize single and multiple acetyllysine on the histone H4 tail. However, the preferred ligands of the ATAD2 bromodomain have not been well characterized, and how adjacent acetyllysine modifications modulate the binding activity of the ATAD2 bromodomain is unknown. We hypothesized that the presence of these nearby modifications would impact histone recognition by the ATAD2 bromodomain. We carried out a systematic screen of potential post translationally modified histone ligands with the ATAD2 bromodomain using newly developed dCypher technology. We further characterized the ligands identified using biophysical techniques including isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) to outline the binding affinities of multiple mono- and di-acetylated histone ligands, and identified residues involved in specific interactions with each histone ligand. Lastly, we solved a high-resolution X-ray structure of the ATAD2 bromodomain in complex with the histone H4K5ac ligand, which displayed new contacts with the N-terminus of histone H4. Our results indicate that the ATAD2 bromodomain is able to distinguish between very similar acetyllysine modifications on the histone H4 tails to recognize specific PTM combinations, and further elucidates the molecular mechanisms targeting the ATAD2 bromodomain to chromatin.

DIFFERENTIAL REGULATION OF NEUROTROPIC AND CARDIAC VIRUS INFECTIONS BY THE C-REL TRANSCRIPTION FACTOR

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Herpes simplex virus 1 (HSV-1) encephalitis is an acute neuroinflammatory condition of the brain and is the most common form of sporadic viral encephalitis. In contrast, coxsackievirus B3 (CVB3) can establish productive infection in the heart and can result in lethal cardiomyopathy. Both diseases are strongly influenced by host genetics, where susceptibility to infection depends on the protective or pathological role of tissue-resident cells and infiltrating immune cells. In a chemical mutagenesis screen for HSV-1 susceptibility, we have identified a truncating mutation in the *Rel* gene (Rel^{C307X}), encoding for the NF-kB transcription factor subunit c-Rel, as a cause of lethal encephalitis. Upon HSV-1 infection, the hindbrains of susceptible Rel^{C307X} mice exhibited high viral replication, inflammation, and cell death, reflecting a defect in cell-mediated immunity. Conversely, Rel^{C307X} mice controlled CVB3 viral replication better than wild-type littermate mice and were more resistant to lethal heart infection. To address the divergent role of the Rel^{C307X} mutation in the regulation of these diseases, we performed dual host-pathogen RNA sequencing on infected Rel^{C307X} brainstem and heart tissue. Two days prior to HSE symptom onset in the brain, the Rel^{C307X} transcriptional profile was characterized by elevated viral RNA transcription and by excess interferon-dependent and inflammatory gene expression, predictive of later infiltration of pathological T lymphocytes and myeloid cells that drive fulminant HSE. Yet in the CVB3-infected heart, the Rel^{C307X} mutation provided for an improved control of early viral

transcription, and a later reduction in inflammatory gene expression; these effects were independent of interferon signaling, suggesting that heart-infiltrating T lymphocytes and myeloid cells were attenuated in their ability to cause pathological damage to the heart, and allowed the virus to be efficiently cleared. Ultimately, our findings support a role for the c-Rel transcription factor as a key regulator of inflammation during viral infection and highlight that the intricate balance of a controlled immune response is critical in resolving infection and avoiding pathology. This work also provides a framework, through the combination of host-pathogen transcriptomic profiling with flow cytometric analysis, to evaluate the effect of single gene defects in specific tissue contexts, which often vary in their capacity to support viral infection and tolerate inflammatory responses.

CIS-REGULATORY HUBS CONSTITUTE A POWERFUL MODEL TO UNDERSTAND THE IMPACT OF 3D ORGANIZATION IN SCHIZOPHRENIA

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The cis-regulatory modules (CRMs) are noncoding regulatory regions, playing a crucial role in the regulation of transcription and the emergence of complex phenotypes. Recent single-cell multi-way analyses show that several CRMs and genes locally co-interact through 3D contacts, building hubs. Despite the importance of CRM hubs in gene regulation, their precise implication in complex diseases such as schizophrenia remains unclear. In the present study, we model cis-regulatory hubs (CRHs), using available Hi-C data in neurons derived from induced pluripotent stem cells and the activity-by-contact model linking active enhancers to promoters. Comparing CRHs to either equivalent tissue-specific or non-tissue-specific structures, we showed that they constitute functional organization with pathological implications in schizophrenia. Firstly, we defined CRHs as active structures, associated with gene activity, where genes are mainly co-regulated by several regulatory elements. Indeed, genes inside CRHs by sharing common distal elements converge to common biological process, suggesting that CRHs are biologically functional. Then, we assessed the relevance of CRHs in schizophrenia using H-Magma. Considering the noncoding SNPs in 3D contact with genes, we found an enrichment in schizophrenia-associated genes within CRHs compared to genes outside (OR=1.81). In addition, schizophrenia-associated genes included in CRHs are characterized by higher expression levels, a higher proportion of active regulatory elements, with a tendency to build smaller hubs. Next, using the linkage disequilibrium score regression, to assess the portion of heritability explained by CRHs, we showed that CRHs explain more heritability than non-tissue-specific elements, with enrichment of 3 against 0.43 on average. Moreover, we also observed up to 11-fold enrichment in schizophrenia heritability compared to equivalent tissue-specific elements. This result is supported by schizophrenia-associated SNP enrichment (OR=1.29). Finally, we found that small CRHs, by connecting fewer regulatory elements explain a larger portion of schizophrenia heritability than medium or large CRHs. CRHs are the first computational-based attempt to build networks of promoters and distal regulatory elements, capturing indirect interplay with phenotypic implications. Our results demonstrate that CRHs by providing functional structures in neurons constitute a useful model for understanding the 3D organization between CRMs and genes involved in the emergence of complex phenotypes such as schizophrenia.

MEQTL AND NCRNA EQTL ANALYSES IN DEPRESSION

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Depression is the worldwide leading cause of disability, which may lead to suicidal behavior, if it is left undiagnosed and untreated. Studies suggest that gene-environment interaction contributes to molecular mechanisms of the differential risk for depression. DNA methylation (DNAm) and non-coding RNA (ncRNA) are epigenetic marks playing a crucial role in the regulation of synaptic formation and function across lifespan. The aim of this study was to investigate if the environmentally modifiable factors, DNAm and ncRNA expression, are associated with proximal genetic variation and depression risk.

We performed DNAm quantitative trait locus (meQTL) analysis of the 102 single-nucleotide polymorphisms (SNPs), previously identified in the most recent and largest genome-wide meta-analysis of depression. A total of 64 SNP-CpG pairs (padj. < 0.05) were identified and replicated in 435 healthy individuals. We further tested functional associations between the DNAm levels at the identified CpGs and expression levels of the associated genes and depression in blood and brain. Separately, depression-related SNPs were investigated for the eQTL effect on the nearby ncRNA. Our findings highlighted that SNPs at the HACE1 and SHANK2 genes may play a regulatory role in depression etiology via altered DNAm levels and miRNA expression.

Our findings support epigenetic differences due to genetic factor in depression in a multi-tissue (blood and brain) and multi-layered (genetic, epigenetic, transcriptomic) approach. This study gives more knowledge of molecular mechanisms in depression and suggest that modifiable biological factors should be evaluated as targets for prevention and treatment.

MH2A1.1-PARP1 INTERACTIONS ARE RELEVANT IN MEMORY CONSOLIDATION

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The formation of long-lasting memories requires learning-induced changes in gene expression, which is tightly regulated by epigenetic factors that modulate DNA accessibility. Histone variants were recently identified as novel regulators of memory, whereby the histone variant H2A.Z functions as a memory suppressor, but the role of other H2A variants in memory formation is unclear. Our lab recently identified macroH2A (mH2A), a structurally unique H2A variant bearing a large macrodomain, as another novel regulator of memory. mH2A is encoded by two genes, *H2Afy* and *H2Afy2*, encoding mH2A1 and mH2A2 respectively. Only mH2A1 is dynamically regulated by learning, and its eviction from hippocampal chromatin predicts learning-induced upregulation of gene expression. Furthermore, hippocampal mH2A1 depletion, but not mH2A2 depletion, impairs hippocampal-dependent memory, suggesting gene-specific roles of mH2A variants in memory. Here, we further investigate the role of mH2A1 in memory by differentiating between two splice variants, mH2A1.1 and mH2A1.2, which bear structurally distinct macrodomains. Only the mH2A1.1 macrodomain binds poly(ADP)-ribose (PAR) and interacts with poly(ADP-ribose)polymerase 1 (PARP1), which is itself implicated in learning and memory. We showed that PAR levels and PARP1 expression increase in response to neuronal activity and to an associative learning event, and that PARP1 is upregulated by mH2A1 depletion. Moreover, impairments in fear memory produced by mH2A1.1 depletion are rescued by PARP1 co-depletion, suggesting that mH2A1.1-PARP1 interactions regulate memory. These studies are the first to investigate isoform-specific effects of mH2A1 on hippocampal-dependent memory and transcription and characterize the functional relevance of PARP1-macrodomain interactions in these processes.

HISTONE DEACETYLASE 3 REGULATES MICROGLIA GENE EXPRESSION AND FUNCTION

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As the resident immune cell of the central nervous system (CNS) microglia play a critical role in establishing neural circuitry in the developing brain and actively surveying immune function throughout life. Patterns of microglia gene expression which underly microglia immune response are regulated in part by epigenetic modifying enzymes such as histone deacetylases (Hdac), which canonically remove histone acetyl groups to repress gene expression. How these epigenetic-modifiers regulate microglial gene expression and immune response remains poorly understood. We hypothesize that Hdac3 is involved in the baseline repression of acetylation and expression of pro-inflammatory genes, which upon immune activation releases, allowing for pro-inflammatory gene expression. To investigate this paradigm we used an Hdac3-specific inhibitor RGFP966 to selectively block Hdac3 enzymatic activity and a pan-Hdac inhibitor SAHA that blocks all class I Hdacs. Primary rat microglia cultures were incubated for 1-hour with the Hdac inhibitor drug followed by 3-hour lipopolysaccharide (LPS) stimulation. Using immunofluorescent staining, we observed that both RGFP966 and SAHA enhanced levels of histone 3 lysine 27 acetylation (H3K27ac), an epigenetic marker of enhancer activation, and H3K9ac, an epigenetic marker of promoter activation. Using RT-qPCR we measured changes in gene expression of interleukin-1 beta *(IL1-b)* and chemokine ligand-16 *(Cxcl16).* Interestingly these responses differed between gene as well as Hdac inhibition, demonstrating a unique role of Hdac3 in microglia immune response. We investigated functions of microglia using two assays; a fluorescent phagocytosis assay and a nitric oxide (NO_2^-) production assay, both supporting activation of primary microglia with LPS treatment. Our next studies will investigate Hdac3 occupancy at candidate genes *IL1b* and *Cxcl16* to assess the direct role of Hdac3 and associated transcription factors. These results have important implications for the understanding of how epigenetic modifications contribute to the tightly regulated microglia gene expression patterns under homeostatic and inflammatory conditions. The findings have implications for the use of Hdac inhibitor drugs as novel neuro-immune modulatory therapeutics for brain disorders.

INVESTIGATING THE ROLE OF THE ANAPHASE PROMOTING COMPLEX IN BREAST CANCER PROGRESSION

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High mortality rates in triple negative breast cancer (TNBC) are attributed to multiple drug resistance (MDR) causing innate aggressiveness compared to estrogen receptor positive cancers. Reversing MDR in TNBC requires targeting chromatin and chromosome instability, a major driver of the disease. Activation of the Anaphase Promoting Complex (APC), a highly conserved ubiquitin ligase, extends yeast lifespan and protects against MDR in human cancer cells. The APC targets many substrates for proteasomal degradation through polyubiquitination during the M and G1 phases of the cell cycle and plays a role in mitotic chromatin assembly and histone modification. Therefore, increasing APC activity may push malignant cells through premature mitosis while harboring high levels of chromosome instability, resulting in mitotic catastrophe and death of MDR cells. When combined with DNA damaging chemotherapy, premature progression of these cells through mitosis could be even more debilitating. Therefore, we hypothesize that increasing the activity of the APC through novel binding and activating peptides will provide genome protection and slow tumor growth while behaving synergistically with chemotherapy agents. This project will extend the current knowledge base of the APC and its relation to TNBC while attempting to counter MDR and create a less toxic more effective form of treatment to improve patient outcomes.

SEX-SPECIFIC PROFILES OF M6A RNA METHYLATION IN THE BRAIN OF INDIVIDUALS WITH MAJOR DEPRESSIVE DISORDER

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Introduction: Females are twice as likely to be diagnosed with Major Depressive Disorder (MDD); however, males are 3.5 times more likely to die by suicide. This is a striking example of sex differences in MDD, and mounting evidence suggests that it may be driven by sex-specific molecular mechanisms. Epigenetic mechanisms, which are altered in response to environmental factors, are known to be involved in the pathophysiology of MDD; however, little is known about the impact of the epitranscriptome. In recent years, RNA modifications have emerged as a dynamic and crucial mechanism in the post-transcriptional regulation of gene expression. Among the 150 known RNA modifications, N6-methyladenosine (m6A) is the most abundant and reversible RNA modification in mammalian messenger RNA (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. This study aims to describe the landscape of m6A in the human brain and to identify changes that may occur in the context of MDD.

Methods: First, the postmortem stability of m^6A and the influence of age, RNA Integrity, and pH on global m^6A levels were investigated in human postmortem brain tissue. Next, we optimized a low-input m6a-seq method for human postmortem brain tissue and confirmed that the m6a-peaks are enriched in the known m6A GRACH motif and near 3'UTR and stop codon as suggested by the previous study.To investigate the role of m6A in MDD, the ventromedial prefrontal cortex was obtained from male and female MDD and healthy control subjects. We performed m6A-seq and RNA-sq to investigate m6A at transcripts levels and the impact of m6A on gene expression.

Results: Our results suggest that PMI does not significantly influence global m^6A levels, and m^6A is relatively stable in the postmortem brain. We identified ~25,000 m6A peaks in the human brain, and these peaks were enriched in genes related to neuronal and synaptic regulation. Moreover, our results show a distinct m6A profile in MDD and control, with a little overlap between males and females.

Conclusion: Our results provide insight into the molecular mechanisms involved in sex-specific transcriptomic signature of MDD. This project will help us understand the role of m6A in stress-related psychiatric disorders and will serve as a much-needed example of sex-specific analysis in psychiatric research.

MOLECULAR CONTROL OF GENOME ARCHITECTURE

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The nuclear lamina (NL) has a major regulatory role in higher order genome organization and gene regulation, as inactive heterochromatin (HC) is tethered to the periphery of the conventionally organized nuclei. Indeed, more than 400 pathological mutations in LMNA gene, which encodes for lamin A, a key protein component of the NL, have been associated with a wide spectrum of diseases called laminopathies, affecting muscles, skin, bones, adipose tissue and peripheral nerves. Lamin A mutations are associated with the premature and normal aging process as well, with abnormal morphology and partial loss of peripheral heterochromatic layer as distinct hallmarks of affected nuclei. As the specific contribution of disrupted 3D genome organization to disease pathogenesis remains unclear, we aim to study molecular structure, function and significance of lamin A, one of the key players in spatial organization of chromatin. We propose to take advantage of the inverted architecture of mouse rod photoreceptor nuclei, which is devoid of any tethering proteins. Using electroporation, cloned lamin A constructs have been transfected in the rod nuclei, serving as an in vivo assay for tethering sufficiency. By utilizing Airyscan confocal super- resolution microscopy, in our published research, we discovered lamin A is sufficient for heterochromatin tethering to the NL and restoring the conventional nuclear architecture. We hypothesize that a unique lamin A C-terminus binds heterochromatin to facilitate genome compartmentalization in the conventional nuclei. With the help of ImageJ automated and unbiased image analysis macro for quantitating changes in heterochromatin distribution, we are going to identify the molecular mechanism of lamin A tethering function and map lamin A heterochromatin binding domain/s. We will evaluate the purpose of lamin A tethering function by inspecting how lamin A impacts genome accessibility, epigenome and transcriptome, using ATAC-seq, CUT&Tag-seq and scRNA-seq, respectively. We will integrate this information to develop a model explaining how lamin A organizes the genome mechanistically and functionally. This could help in elucidating whether and how peripheral heterochromatin loss contributes to pathological states and aging. Our findings might impact the field of human disease genomics and bring novelty in the field of genome biology.

PRECLINICAL MOUSE MODELS OF ACUTE MEGAKARYOBLASTIC LEUKEMIA ENABLE THE IDENTIFICATION AND TESTING OF NEW DRUG VULNERABILITIES

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RATIONALE The CBFA2T3-GLIS2 fusion oncoprotein is frequently recovered (> 20%) in paediatric, non-down syndrome acute megakaryoblastic leukemia (non-DS AMKL; AML-M7 FAB classification). Furthermore, current standard protocols used for the treatment of AML have proven unsuccessful in children harboring the CBFA2T3-GLIS2 fusion, hence showing the poorest survival rate amongst all pediatric AMKL. This is likely due to the lack of understanding of the specific molecular mechanisms by which CBFA2T3-GLIS2 drives tumorigenesis.

PURPOSE OF THE STUDY We generated a *CBFA2T3-GLIS2*-driven preclinical mouse model of AMKL to ***i)*** investigate genes/pathways involved in the development of the disease, ***ii)*** identify novel therapeutic targets leading to growth suppression of CBFA2T3-GLIS2+ AMKL cells and ***iii*)** develop and trial targeted therapies *in vivo*.

RESULTS Our *de novo* model of CBFA2T3-GLIS-driven AMKL mimics most aspects of human disease, principally characterized by a drastic accumulation of megakaryocytic blasts in the peripheral blood and bone marrow, as well as infiltration into extramedullary organs such as the spleen. Furthermore, blasts in the spleen and bone marrow express many megakaryocytic (CD41, CD61, CD42, factor VIII) and immature megakaryocyte progenitors (c-Kit+/CD41+), and the morphology of bone marrow and spleen cells is reminiscent of megakaryoblasts. Moreover, genome-wide RNA sequencing (RNA-seq) of leukemic blasts highlighted genes that are overexpressed in paediatric AMKL patients (ITGA2B/CD41, BMP4, GLIS2, ERG, MPL), while gene set enrichment analysis corroborated with human AMKL transcriptional signatures, with increased JAK-STAT and RAS-MAPK signaling genes. RNA-seq of murine fetal liver cells expressing individual gene members of the fusion revealed that the GLIS2 moiety (member of the GLI family of transcription factor) drives the megakaryocytic identity of leukemic blasts. Furthermore, treatment of human CBFA2T3-GLIS2+ AMKL cell lines and murine leukemic cells - isolated from the bone marrow of sick mice - with a GLI inhibitor led to apoptosis while sparing normal bone marrow cells. This GLI inhibitor is currently being tested *in vivo* using our preclinical AMKL mouse model and will further be tested in patient-derived xenografts.

CONCLUSION The prognosis of children with this fusion is significantly worse when compared to CBFA2T3-GLIS2 negative patients in general. Overall, our model now serves as a tool to study molecular pathways involved in the disease and allows the identification of genes/pathways that may be targeted by therapeutic drugs. In the end, this project will directly support the advance of optimized and efficient clinical protocols specific to this life-threatening subset of AML.

A TBP-INDEPENDENT MECHANISM FOR RNA POLYMERASE II TRANSCRIPTION

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Transcription by RNA Polymerase II (Pol II) is initiated by the hierarchical assembly of the Pre-Initiation Complex (PIC) 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. Surprisingly, Pol II transcriptional induction through the Heat Shock Response occurs normally in the absence of TBP. In contrast, acute TBP depletion severely impairs initiation by RNA Polymerase III. Lastly, we show that the binding of the PIC is perturbed upon the loss of TBP, yet Pol II-mediated transcription remains unchanged. Taken together, our findings reveal an unexplored TBP-independent process in mESCs that points to a diversity in Pol II transcription initiation mechanisms.

INFERRING COPY NUMBER VARIATION IN CANCER SINGLE-CELL CHROMATIN ACCESSIBILITY DATA WITH COPY-SCAT

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Single-cell accessibility datasets in clinical cancer specimens are highly heterogenous mixtures of neoplastic and stromal cells, and accurate delineation of tumour and non-tumour cells can be challenging in many tumour types. Copy number alterations are common across many different cancer types and represent one potential way to distinguish tumour from non-tumour cells in single-cell experiments. We have developed an R package, Copy-scAT, which identifies areas of putative extrachromosomal amplification and large-scale CNVs in cancer data sets. We validate our method using data from adult

and pediatric glioblastoma, and multiple myeloma. In addition, we explore the influence of subclonal genetic and epigenetic phenotypes in adult glioblastoma, finding intratumoral epigenetic differences at the subclonal level. Copy-scAT enables improved delineation of neoplastic and non-neoplastic cells and clonal dynamics in single-cell accessibility cancer datasets.

ASCL1 ACTIVATES NEURONAL STEM CELL-LIKE LINEAGE PROGRAMING THROUGH REMODELING OF THE CHROMATIN LANDSCAPE IN PROSTATE CANCER

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Background: Second generation AR pathway inhibitors (ARPIs) such as Enzalutamide (ENZ) are highly effective in castration resistant prostate cancer (CRPC). However, they play a role in emergence of a more aggressive, AR-independent phenotypes including treatment induced neuroendocrine prostate cancer (t-NEPC). With the exception of genomic alterations to RB1 and TP53, few other genetic differences are observed between CRPC and NEPC; suggesting an epigenetic dysregulation underlining this conversion.

Method: In order to capture the evolution of CRPC to NEPC, we measured changes in chromatin accessibility of CRPC cells upon exposure to ENZ and in NEPC cell lines via ATAC-seq. We performed RNA-seq from matched treatment. In addition, we successfully conducted ASCL1, H3K27me3 and EZH2 ChIP-seq.

Result: Using ATAC-seq we identified ASCL1 motif becoming accessible as early as 3 days, with continued enrichment at 10 days post ENZ-treatment as well as in NEPC cell-line models. ASCL1 expression and activity is significantly upregulated in NEPC cell lines and patient tumors. Using our unique model of tNEPC, we showed that knockdown of ASCL1 causes extensive chromatin reorganization leading to reduced expression of neuronal and plasticity markers and overall abolishment of the NEPC program. Combining ASCL1 and H3k27me3 ChIP-seq discovered that in NEPC models close to 40% of ASCL1 binding sites overlap with H3k27me3. Loss of ASCL1 function dysregulated Polycomb repressor complex 2 (PRC2) activity (loss of H3k27 methylation). This results was captured across multi-omic analysis integrating RNAseq, ChIPseq and protein expression. Interestingly this global loss of H3K27me3 occurred without any affect on the expression of PRC2 complex members. Cell fractionation, confocal microscopy and EZH2 ChIP-seq identified loss of EZH2 binding to the chromatin as the likely cause of the H3K27 demethylation. Pheno-copying EZH2 inhibition, this loss of H3K27me3 reactivated luminal programing, potentially re-sensitizing these cells to further treatments. Altogether, our result suggesting that ASCL1 may drive early transcriptional and epigenetic reprogramming through the PRC2 complex, therefore, facilitating the emergence and maintenance of NEPC. Conclusion: In closing, we report a novel role for pro-neuronal transcription factor ASCL1 in modulating the chromatin dynamics to support a plastic lineage by orchestrating early chromatin events and regulatory networks that determine a neuronal stem cell-like lineage commitment. In the treatment-resistant, high plasticity state inhibition of ASCL1 reverses the lineage switch to epithelial-luminal, providing a potential for targeting these highly aggressive tumors. Similar to NEPC, a subset of glioblastoma and small cell lung cancers are defined by elevated expression of ASCL1. This work provides much-needed insight into ASCL1 function and dependency that together nominates ASCL1 as a bona fide clinical target.

INVESTIGATION OF METHYLATION STATUS IN RELATION TO THE PRESENCE OF BORRELIA BURGDORFERI IN IXODES SCAPULARIS (BLACK-LEGGED TICKS)

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Ticks, arthropod carriers of *Borrelia burgdorferi* and other bacterial, viral and protozoan pathogens are largely responsible for the spread of Lyme disease. The increasing prevalence of Lyme disease globally has led to further research into the mechanisms and causes of the disease and spread of infection. Underlying mechanisms of behavioral and physical modifications of ticks, specifically *Ixodes scapularis* that result in increased fitness due to the tick infection status are not fully understood. The bacterium *Anaplasma phagocytophilum* is recognized to alter the physiology of ticks through epigenetics mechanisms; similarly, we postulated that changes in DNA methylation may also result from the interaction of *Ixodes scapularis* and *Borrelia burgdorferi.* This study seeks to determine if the methylation status in tandem repeat regions *Ixodes scapularis* repeats (ISRs) differs between *Borrelia burgdorferi* infected and uninfected *Ixodes scapularis* ticks. DNA was extracted from female *Ixodes scapularis* ticks, both infected and not, as verified by nested PCR and used for Methylated-DNA immunoprecipitation followed by quantitative PCR. Primers were designed to target ISR regions 1-3. Preliminary results suggest that *Borrelia burgdorferi* may modify the levels of DNA methylation in *Ixodes scapularis* ticks. This study provides a basis for future epigenetic studies on the epigenetic interactions between pathogens and possible phenotypic changes in their arthropod vectors.

CHARACTERIZING THE ROLE OF TDG IN FXR-DEPENDENT SIGNALLING

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DNA methylation is an epigenetic mechanism that is essential for growth and development. The 5-methylcytosine (5mC) mark can be reversed through active demethylation, in which 5mC is converted to an unmodified cytosine through an oxidative pathway coupled with base excision repair (BER). The BER enzyme Thymine DNA Glycosylase (TDG) plays a key role in active demethylation by excising intermediates of 5mC generated by this process. The embryonic deletion of TDG in mice is lethal at E11.5, suggesting that TDG's role in maintaining epigenetic stability is required for embryonic development. To bypass this embryonic lethality, we have generated a conditional TDG knockout mouse model (TDGCKO; TDG -/fl UBC-cre/ERT2+) in which TDG is deleted eight weeks post-partum. TDG_{CKO} mice spontaneously develop late-onset hepatocellular carcinoma (HCC) associated with diabetic symptoms such as increased body weight, hyperglycemia, and bile acid (BA) overload. This phenotype is largely attributed to a defect in the Farnesoid X Receptor (FXR), a key regulator of BA homeostasis and hepatic glucose homeostasis. Interestingly, FXR_{KO} mice also develop late-onset HCC associated with diabetic symptoms and share a similar transcriptional profile to TDG_{CKO} mice, prompting us to investigate a role for TDG in FXR signalling. To this end, we generated *Tdg*/*Fxr* double-knockout (DKO) mice. We also generated a novel FXR_{KO} mouse model using CRISPR/Cas9, which facilitated the knockout of FXR through a 47-bp deletion event. This deletion event resulted in the formation of a premature stop codon in the ligand-binding domain of FXR, resulting in no FXR expression in the livers of FXR_{KO} mice. The 3-week-old FXR_{KO} mice display impaired bile acid and glucose metabolism. In the DKO mice, neither TDG nor FXR expression were detected based on western blotting. Collectively, these findings demonstrate that our novel FXR_{KO} mouse model phenocopies previous FXR_{KO} mouse models. These mice will provide a unique model to study the mechanism of HCC development and the etiological connection between metabolism and HCC in humans.

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 has recently been shown to function as pioneer transcription factors to modulate the target 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 loss of digits and the loss of expression of HOX13 pioneer activity-dependent targets. We also observe by ATAC-seq that 75% of the HOX13 dependent pioneer sites had lost their 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. 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 remains 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, which we hypothesize may be the consequence of compromised HOX13 pioneer activity.

REGULATION OF THE EPIGENOME AND TRANSCRIPTOME BY ATRX IN MOUSE ASTROCYTES IS REQUIRED FOR MEMORY PROCESSES

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Astrocytes are glial cells of the central nervous system that regulate synapse formation, maturation and elimination. Defective astrocytes can alter synaptic transmission and contribute to neurological disease. ATRX is a chromatin remodeler, and mutations in the *ATRX* gene lead to syndromic and non-syndromic intellectual disability. However, there is little understanding of its functions in different brain cell types. To investigate the role of ATRX in specifically in astrocytes, we used a conditional and inducible Cre/loxP system in mice (*Atrx*$^{f/y}$;*GlastCre*^{ER}). Tamoxifen was intraperitoneally injected daily from postnatal day 10 to 12 to induce *Atrx* deletion in astrocytes prior to their maturation phase (ATRX astrocyte inducible knockout, or ATRX aiKO mice). Immunofluorescence staining indicates that ATRX is absent in approximately 50 % of the astrocytes in the cortex and hippocampus of ATRX aiKO mice. Behaviour tests reveal that ATRX aiKO mice exhibit long-

term recognition and spatial memory deficits compared to controls. *Ex vivo* electrophysiology measurements, dendritic branching, and spine morphology of CA1 neurons revealed reduction in synaptic function of hippocampal CA1 neurons but normal morphology. Astrocyte nuclei were purified using fluorescence activated nuclei sorting (FANS) and used for RNA-seq and chromatin accessibility assays (ATAC-seq). Pathway analyses show enrichment of differentially expressed genes linked to cytoskeletal biology and synapse organization. Integration with ATAC-seq data identified candidate pathways and targets responsible for astrocyte-mediated neuronal dysfunction linked to memory deficits, such as brain-derived neurotrophic factor release and cannabinoid receptors. Collectively, these findings demonstrate that ATRX is required in astrocytes for normal synaptic transmission in neighbouring hippocampal neurons, thus contributing to long-term memory processes.

HISTONE ACETYL LYSINE RECOGNITION BY THE BRPF1 BROMODOMAIN

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BRPF1 is a large multidomain protein consisting of three chromatin reader domains – a double PHD and zinc finger assembly (PZP), the bromodomain (BRD) and a chromo/Tudor-related Pro-Tyr-Tyr-Pro (PWWP) domain. Bromodomains (BRD) are evolutionary conserved protein interaction modules that are known 'readers' of the acetyllysine modifications on histones. BRPF1 is a key regulatory subunit of the MOZ HAT complex and is known to direct MOZ HAT complex to the chromatin through recognition of acetylated histones. The human monocytic leukemia zinc-finger protein (MOZ) histone acetyltransferase (HAT) complex acetylates free histones H3, H4, H2A and H2B in vitro and is directly involved in hematopoiesis and the development and maintenance of the hematopoietic stem cells (HSCs). The MOZ/HAT functions as a quaternary complex containing BRPF1, the inhibitor of growth 5 (ING5) and the human Esa1- associated factor 6 homolog (hEAF6). Despite the importance of multiple modifications in epigenetic signaling pathways, little is known about how combinations of these modifications are recognized and influence the histone binding by BRPF1, which is known to direct the HAT activity of MOZ. The recognition of acetyllysine by the bromodomain is crucial to activation of gene transcription making BRD a promising drug target in various diseases including cancer, neurological disorders, and cardiovascular diseases.

We have previously identified that the BRPF1 bromodomain can recognize multiple acetyllysine marks on the histone tails (Poplawski et al., 2013). Here we identify that the BRPF1 BRD has a preference for di-acetyllysine histone H4 tail ligands. ITC confirmed that the BRPF1 BRD binds with the highest affinity to histone H4K5acK8ac and H4K5acK12ac peptides. Solution NMR spectroscopy was then used to map the residues lining the BRPF1 bromodomain binding pocket involved in interaction with the di-acetyllysine histone H4 tail ligands. Mutational analyses and AUC experiments further emphasize the role of BRPF1 BRD residues involved in the recognition of histone ligands.

Through our study we want to highlight the importance of cross-talk between various histone post-translational modifications and how it may regulate BRPF1 activity. These combinations of PTMs can either promote or inhibit the affinity of BRPF1 to the histones and in turn could affect how BRPF1 recruits MOZ HAT to the chromatin. Understanding the molecular mechanisms driving these interactions may provide therapeutic interventions for the treatment of various diseases.

LINEAGE-SPECIFIC ROLE OF SMARCD1 SUBUNIT OF SWI/SNF CHROMATIN REMODELING COMPLEXES DURING LYMPHOPOESIS

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During hematopoiesis, stem cells (HSCs) either self-renew or differentiate into all mature blood cell-types through successive rounds of binary cell fate decisions. The prevailing model of hematopoiesis predicts a step-by-step model of lineage differentiation in which HSCs first give rise to multipotent progenitors that subsequently differentiate into myeloid and lymphoid restricted progenitors. Although key transcriptional pathways controlling hemopoietic development are beginning to be revealed, detailed molecular mechanisms explaining how HSCs and early progenitors are initially primed to the different hemopoietic cell lineages are still lacking. Work from our laboratory indicates that combinatorial assembly of SWI/SNF chromatin remodeling complexes is a key epigenetic mechanism that governs HSC self-renewal and lineage cell fate decisions. Transcriptomics analyses revealed that expression of the three Smarcd family members of the SWI/SNF complex diverge during hematopoietic differentiation indicating a potential differential role during this process. More particularly the Smarcd1 subunit expression is enriched in hemopoietic stem/progenitor and early lymphoid progenitor cells while nearly absent in other lineages suggesting its involvement in lymphoid differentiation. Using a conditional knock-out mouse model, we confirmed that Smarcd1 is required for early lymphopoiesis processes. Our transcriptomics analyses in sorted multipotent progenitors revealed an essential role for Smarcd1 to prime essential lymphoid genes like Il7r, Rag1, Rag2 and Dntt in those uncommited progenitor cells. Moreover, we found that Smarcd1 transcriptomic signature highly correlates with the bHLH transcription factor E2A an essential regulator of lymphopoeis. Mechanistically, we showed that Smarcd1 physically interacts E2A and is required for chromatin remodeling at a set of active enhancers in proximity of primed

lymphoid genes and co-bound by E2a and the SWI/SNF complex. Impairing the interaction between Smarcd1 and E2A through targeted mutagenesis of the Smarcd1 SWIB domain inhibits lymphoid lineage determination and the emergence of lymphoid-primed multipotent progenitors confirming the direct collaboration of these two factors. Altogether, this work revealed that Smarcd1 is a master regulator of lymphopoiesis that remodels chromatin to allow the recruitment of lineage-specific transcription factors at essential loci for lineage specification.

CATEGORIZING SYNOVIAL SARCOMAS BASED ON EPIGENOMIC LANDSCAPE

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Background: Synovial sarcoma (SS) is an aggressive soft-tissue malignancy characterized by a pathognomonic chromosomal translocation leading to the production of SS18-SSX, a fusion oncoprotein. Previous research shows that SS18-SSX associates with BAF, a chromatin remodelling complex, suggesting epigenetic mechanisms drive this cancer. We hypothesize that SS can be sub-grouped based on epigenomic state and that these subgroups relate to disease severity.

Methods: We profiled 31 cases of primary human SS using chromatin immunoprecipitation sequencing (ChIP-seq) for histone modifications (specifically H3K27ac, H3K4me3, H3K4me1, H3K27me3, H3K36me3, H3K36me2, H3K9me3), RNA-seq for transcriptomes, and whole genome bisulfite sequencing (WGBS) for DNA methylomes. Publicly-available cell line data were obtained for comparison.

Results: Unsupervised hierarchical clustering of genome-wide histone ChIP-seq density for the transcriptionally active marks (H3K27ac, H3K4me1, H3K4me3) reveals two major SS sub-groups. Enhancers (regions marked by H3K27ac) from SS Group 1 tumors show lower levels of BAF binding compared to SS Group 2 enhancers. Using published isogenic cell line models, Group 2 enhancers also show greater overlap with binding sites of non-oncogenic BAF complexes (lacking SS18-SSX) compared to oncogenic BAF complexes (containing SS18-SSX). Genes associated with Group 2 enhancers are expressed at lower levels in SS compared to other soft-tissue sarcomas, suggesting Group 2 tumors express SS signature genes at lower levels than Group 1 tumors. Clinical data show that Group 2 tumors are lower grade tumors. Treating SS cells with quisinostat, a histone deacetylase inhibitor, leads to cell death and increases the expression of Group 2 enhancer genes.

Conclusion: SS can be sub-grouped into two distinct classes based on epigenomic state, associated with differences in BAF activity. Using a histone deacetylase inhibitor pushes cells into a phenotype that resembles the less aggressive sub-group, supporting its value as a therapeutic strategy in SS.

ANALYZING SEQUENCE FEATURES OF MEMBRANE LESS ORGANELLES TO UNDERSTAND ABERRANT LIQUID-LIQUID PHASE SEPARATION IN CANCER

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Background and Purpose: Liquid-Liquid Phase Separation (LLPS) is a phenomenon where proteins and/or nucleic acids concentrate into a dense phase resembling a liquid droplet, which is surrounded by a dilute phase. Throughout the cell, LLPS is responsible for forming membrane less compartments such as stress granules, constitutive heterochromatin, nuclear speckles, PML bodies, and Cajal bodies. These membrane less organelles have critical functions that are deregulated in cancer. Aberrant LLPS has established roles in the pathogenesis of neurodegenerative disorders, but its role in cancer is just emerging. Although membrane less compartment formation by LLPS is typically driven by intrinsically disordered regions (IDRs), other amino acid sequence features also play significant roles. In this context, we wanted to determine if sequence features can distinguish phase separated compartments. To that end, we analyzed the pericentromeric heterochromatin, stress granule and nuclear speckle compartments, because they have broad roles in cellular homeostasis and cancer.

Methods: Using published proteomic datasets, we determined the constituents of pericentromeric heterochromatin, stress granule, and nuclear speckle compartments. Next, we manually extracted the sequences of 187 heterochromatin proteins, 134 stress granule proteins, and 182 nuclear speckles proteins. We used LocalCider and Python to write functions that determined 47 different amino acid sequence parameters from each of the protein sequences. We then used R studio to perform principal component analysis (PCA) on the 47 different sequence features across the three protein datasets.

Result: In principal component analysis, pericentromeric heterochromatin, stress granule and nuclear speckle compartments do not form distinct clusters. Nevertheless, protein datasets did form clusters of weak, intermediate, and strong polyampholytes according to their charge properties. Within this classification, the nuclear speckle dataset contained higher levels of proteins in the strong polyampholytes category, suggesting they may a key driver of speckle compartment assembly and function.

Conclusion: As PCA shows the principal direction in which the data varies, the result indicates that that the amino acid sequence feature that differs most between the three compartments is charge property. Nevertheless, classification by charge property is not sufficient to distinguish between the pericentromeric heterochromatin and stress granule compartments. Future work will focus on the biological functions and significance of interactions between the weak, intermediate, and strong polyampholytes within each protein

datasets. Finally, datasets will be analyzed for more complex sequence features, including short linear motifs, to identify potential determinants of compartment specificity.

EPIGENOME-WIDE DNA METHYLATION AND TRANSCRIPTOME PROFILING OF LOCALIZED AND LOCALLY ADVANCED PROSTATE CANCER: UNCOVERING NEW MOLECULAR MARKERS

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Background & Objectives: Significant advances have been made to identify molecular markers for accurately diagnosing the prostate cancer stages between localized prostate cancer (LPC) and locally advanced prostate cancer (LAPC). However, there is a lack of profiling both epigenome-wide DNA methylation and transcriptome of the same patients with prostate cancer at different stages to identify molecular markers. We hypothesize that genes with expression levels that are downregulated in LPC and methylation levels in its promoter regions that are hypermethylated in LPC are the potential gene signature that can be used to distinguish the patients between the LPC and the LAPC.

Materials and Methods: We profiled 10 prostate cancer patients (4 LPC, 6 LAPC) using the Illumina Infinium Human Methylation450 BeadChip (Cheng et al. Clinical Epigenetics (2018) 10:54) and the high-density oligonucleotide Affymetrix human genome array HG-U133 Plus 2.0 (Ribeiro et al. BMC Medicine 2012, 10:108) in the Center for Applied Genomics (Toronto) before. The samples were collected in 2009 by the Portuguese Institute of Oncology, Porto Centre. The data were preprocessed in our previous published studies. We used the Bioconductor "limma" R package to identify differentially methylated CpG sites and differentially expressed genes from the methylation and gene expression data, respectively. We performed an integrative analysis of the microarray gene expression profiles and DNA methylation profiles using LASSO (least absolute shrinkage and selection operator) between each gene and all the CpG sites in its promoter region. We constructed the gene signature from the combination of the association analysis and the differential analysis. We then refined our gene signature using the genetic mutation data obtained from cBioPortal for more than 1500 LAPC and LPC samples from 4 different studies. Using Fischer's exact test, we determined genes that were the most significantly affected by mutations.

Results: From the LASSO-based association analysis, we identified 56 genes presenting significant anti-correlation between the expression level and the methylation level of at least one CpG site in the promoter region (p-value<$5x10^{-8}$). From the differential analysis, we detected 16,405 down-regulated genes and 9,485 genes containing at least one hypermethylated CpG site. We identified 30 genes that showed anticorrelation, down-regulation, and hyper-methylation data. Using the genetic mutation data we determined 6 of the 30 genes showed significant differences (adjusted pvalue<0.05) in mutation frequencies between the LPC and LAPC samples.

Conclusions: In summary, we established an integrative bioinformatic framework to identify differentially expressed genes with an aberrant methylation pattern that may represent novel candidate molecular markers for diagnosing LPC and LAPC.

INVESTIGATING THE ROLE OF BAF53B IN NEURONAL GENE EXPRESSION

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The BAF (BRG1/BRM Associated Factor) nucleosome remodeling complex is critical for proper brain development. As an ATP-dependent chromatin-remodeling complex, the BAF complex can alter histone-DNA interactions facilitating dynamic changes in gene expression by controlling DNA accessibility to the transcriptional machinery. The BAF complex contains up to 15 subunits and subunit switching allows for specialized biological functions. Autism spectrum disorder (ASD) is one of the most prevalent forms of neurodevelopmental disorders, affecting 1 in 66 children in Canada. It is well established that ASD is heritable, however, there are hundreds of genes that have been implicated. Mutations in chromatin remodeling complexes could alter the expression of many genes, potentially explaining the genetic heterogeneity of ASD. The BAF complex has been highly associated with ASD; 12 of the potential 29 subunit genes have been connected to ASD and intellectual disability. Despite the evidence that the BAF complex is critically involved in ASDs, it is still unclear how mutations in BAF actually lead to ASD. A novel, neuron specific version of BAF (nBAF) has emerged as a promising candidate, as it is thought to coordinate the expression of synaptic genes during brain development. Heterozygous deletion of Baf53b, one of the neuron specific subunits of nBAF, disrupts dendritic spine development, synaptic plasticity, and long-term memory in the adult mouse that is reversible with reintroduction of Baf53b. Despite this, it is unclear how changes in gene expression elicited by Baf53b knockout lead to deficits in neuron and synapse development and ultimately ASD. To address this, plasmids with mutations in Baf53b found in patients will be introduced into mouse primary neurons by magnetofection, while simultaneously deleting endogenous Baf53b with a plasmid containing cre-GFP. High resolution microscopy will be used to visualize dendritic morphology and synapse number/morphology in neurons with Baf53b patient mutations compared to wildtype Baf53b. Additionally, a BRG1/BRM ATP inhibitor (BRM014), will be delivered to cultured primary neurons followed by RT-qPCR to explore gene targets potentially regulated by the nBAF complex. This work will expand our understanding of the nBAF complex in neuronal

gene expression and function, providing insight into the etiology of ASD.

THE FUNCTIONAL ROLE OF DNA METHYLATION IN HUMAN TROPHOBLAST STEM CELLS

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DNA methylation, an epigenetic mark mediated by DNA methyltransferases (DNMTs), is required for regulating gene expression and proper embryonic development. De novo methylation occurs in post-implantation blastocyst, generating unique methylation profiles in both the inner cell mass (ICM), which forms embryonic tissue, and the trophoblast, which contributes to the placenta. The placenta plays an essential role in the developmental of fetus during gestation by regulating proper gas/nutrient exchange and generating pregnancy hormones. Alterations in placental DNA methylation are associated with many human placental disorders, including hydatidiform moles and pre-eclampsia. Murine trophoblast stem cells (mTSCs) lacking DNA methylation are shown to survive normally and can contribute to the placenta, indicating that DNA methylation is not essential for the generation of the mouse placenta. To determine the role of DNA methylation in human placental development, we used nucleofection-based delivery of CRISPR/Cas9 to target DNMT1, an enzyme responsible for DNA methylation maintenance, in bulk populations of human trophoblast stem cells (hTSCs). Here, we observe that the DNMT1 Knockout (DNMT1 KO) allele is gradually decreased in bulk populations of DNMT1 KO hTSCs and their DNA methylation profile show a reduction of DNA methylation by day 7 and quickly return to wild-type levels by day 19. These results indicate reduced cell viability in DNMT1 KO hTSCs. Interestingly, DNMT1 KO hTSCs show unchanged cell proliferation activity relative to wild-type hTSCs and cell loss of DNMT1 KO hTSCs is not due to increased caspase-3 activity, an indicator of apoptosis. Loss of DNA methylation led to a loss of nuclear localization of the trophoblast stem cell marker TEAD4, while the majority of upregulated genes are related to germ cell development. We also observed an upregulation of HERV-Fc1 endogenous retrotransposon activity in DNMT1KO hTSCs. In future work, we hope to identify which upregulated genes may be influenced by HERV-Fc1 activity, and which contribute to the reduced cell viability observed in DNMT1 KO hTSCs. Our results show a striking species-specific difference in which DNA methylation is a critical epigenetic mark for human trophoblast cellular homeostasis and will provide fundamental insight on the role of DNA methylation in human placental developmental.

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DNA METHYLATION RESTRICTS GERMLINE SPECIFICATION IN MOUSE EMBRYONIC STEM CELLS.

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DNA methylation is an epigenetic mark associated with gene repression when located on regulatory elements, with an essential role in development. In mammals, DNA methylation profiles are drastically remodeled during early embryogenesis. Following fertilization, gametic DNA methylation patterns are globally erased until the blastocyst stage. This coincides with the emergence of naïve pluripotent embryonic stem cells (ESCs) that can give rise to all lineages. After implantation, while the epiblast stem cells transition from naïve to primed pluripotency, somatic DNA methylation patterns are established through the action of the three DNA methyltransferases DNMT1, DNMT3A and 3B. While somatic lineages mostly maintain these methylation profiles, the germline follows a different trajectory. Indeed, the primordial germ cells (PGCs) that arise from the primed epiblast undergo a global loss of their recently acquired somatic DNA methylation patterns.

Whilst the vital role of DNA methylation for embryonic development is demonstrated by the early lethality of DNMT knock-out mouse embryos, the function played by DNA methylation in the transitioning between pluripotency states, and in the commitment towards somatic versus germline fate, remains unclear.

To understand the role of DNA methylation in early cell fate decisions, we generated DNA methylation-free *Dnmt* triple-KO (TKO) mouse ESCs. We took advantage of differentiation protocols to mirror early development, combined with molecular and functional approaches, to evaluate cellular potency in absence of DNA methylation. We found that DNA methylation is neither required for exiting naïve pluripotency, nor for priming, since pluripotency markers showed expected expression dynamics in the TKO background during epiblast-like differentiation. However, in absence of DNA methylation, ESCs demonstrated extended opportunities for germline commitment. This exciting observation suggests, in opposition to the established dogma, that DNA methylation restricts the developmental window of germline specification, despite being dispensable for the regulation of pluripotency transitioning. I am now studying the underlying mechanisms that promote germline specification in absence of DNA methylation using single-cell analysis, epigenomic profiling and *in vivo* chimera assays. These findings could contribute to shed light onto the nebulous relationship between DNA methylation and the control of cellular identities during development.

A CONSERVED HOXA1-HOTAIRM1 REGULATORY AXIS COORDINATES NEURONAL DIFFERENTIAION

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Long non-coding RNAs (lncRNAs) are rapidly evolving genes that are usually poorly conserved at the transcriptional level. HOTAIRM1 is a lncRNA with a sequence highly conserved across mammals, suggesting it functions in key cellular processes. It is transcribed antisense to *HOXA1* within the *HOXA* cluster, which encodes conserved transcription factors whose highly-regulated, spatio-temporal activation underlies anterior-posterior body patterning during development. Using models for neuronal differentiation, we previously found that HOTAIRM1 is required to physically dissociate neighboring subTADs to prevent premature activation of genes within the *HOXA* cluster. We also found that HOTAIRM1 binds chromatin-modifying complexes that are capable of activating or repressing transcription throughout the genome. We thus wondered if HOTAIRM1 is required for proper neuronal differentiation and which other genes – if any – underlying this process might require HOTAIRM1 function for their appropriate expression.

We find that depleting HOTAIRM1 in cells undergoing neuronal differentiation hampers their proliferation. This led us to consider the expression of transcription factors central to maintaining a cell's pluripotent state and SOX3, an early marker for early neurogenesis with a transient peak in expression. HOTAIRM1 depletion prevents the expected transcriptional activation of *SOX3*. In turn, we observe differential expression of the *SOX2/POU5F1/NANOG* core pluripotent transcription factor network maintaining an undifferentiated state. Given that *HOXA1* and *HOTAIRM1* are transcribed from a common short promoter region and induced to similar levels and kinetics by retinoic acid, we postulated that they might function together along a regulatory axis. We find that the HOXA1 transcription factor can bind the HOTAIRM1 lncRNA by RNA-IP in multiple cell types and species. Interestingly, while HOXA1 depletion only partially recapitulates the perturbed expression patterns of these core pluripotent factors. Together, our data suggests that HOXA1 and HOTAIRM1 physically associate to elicit transcriptional control of downstream targets necessary in mediating between pluripotent and differentiated states during programs exhibiting *HOX* gene activation.

REGULATION OF CHROMATIN STRUCTURE BY ATRX IN MICROGLIA SUPPRESSES CELL ACTIVATION AND ENSURES NORMAL SPATIAL AND RECOGNITION MEMORY

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Mutations in ATRX, a SWI/SNF-type chromatin remodeler, cause an intellectual disability syndrome associated with autistic behaviors in affected boys. Microglia, the resident immune cells of central nervous system, play key roles in regulating the neuronal-microglial interactions, which are necessary for normal synaptic pruning and transmission, neuronal functions, and learning and memory. However, whether microglial ATRX influences learning and memory processes is unknown. To address this question, we generated mice lacking ATRX exclusively in microglia using a tamoxifen-inducible Cre/loxP system. Tamoxifen (2mg/day) was injected daily for 5 days in 45 day-old control and ATRX knockout mice (Atrx miKO), leading to >90% ATRX knockout efficiency in microglia across different brain regions. Chromatin accessibility and the associated transcriptional changes in ATRX-null microglia were investigated using the fluorescent activated nuclei sorting (FANS) followed by RNA-seq and Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq). We identified >32000 differentially accessible regions by ATAC-seq and >2800 differentially regulated transcripts by RNA-seq. Pathway analysis of overexpressed genes revealed enrichment for genes implicated in the cell cycle, innate immunity and inflammation, suggesting that ATRX-null microglia over-proliferate and become activated. This was confirmed by immunofluorescence staining with Ki67 (proliferation marker) and CD68 (activation marker). Behaviour tests of control and Atrx miKO mice revealed anxiolytic effects as well as spatial and recognition memory deficits in the mutant mice. Collectively, these findings demonstrate that ATRX is required in microglia to subdue cell cycle re-entry and cell activation pathways, with consequences on anxiety level and memory processes in the mice. Our study suggests that microglial dysfunction might contribute to intellectual disability in patients harbouring ATRX mutations.

FUNCTIONAL CHARACTERIZATION OF REGULATORY ELEMENTS INVOLVED IN MOUSE CD4+ T CELL DIFFERENTIATION

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The adaptive immune system is an exceptional model for studying complex cellular phenotypes with a high degree of therapeutic significance. CD4+ T helper cells are important mediators of both immunity and autoimmunity. With unique functions, T helper cells support other cell types in clearing of infection or by attenuating an immune response. Their dysregulation is often associated with chronic inflammation, autoimmune disease, and allergy. Major advancements in genetics and genomic technologies have revealed that combinations of extrinsic and intrinsic factors govern T helper cells activation, differentiation, and immune function. Many datasets have detailed the genomic location of thousands of candidate regulatory elements involved in these processes;

however, the functional contribution of those elements remain poorly understood and difficult to study. **Objective:** To empirically measure the activity and function of candidate regulatory elements involved in the differentiation of mouse T helper subsets. This comprehensive functional study will enable the characterization of both the genetic and epigenetic components responsible for regulating gene expression during an immune response. We anticipate these results will lead to the improved design of therapeutic interventions for immune-related disease. **Approach:** We used the high-throughput reporter assay STARR-seq coupled with ATAC-seq to assay the regulatory activity of all open chromatin regions in mouse T helper type 1 (Th1), type 2 (Th2), type 17 (Th17), and T regulatory (Treg) cells *in vitro*. We applied a joint analysis model of multiple data to identify regulatory elements that are common or unique across each subtype. We analyzed sequence features and integrated gene expression and epigenetic datasets to determine what core motifs and chromatin context drive regulatory activity. We applied an orthogonal CRISPR-interference screen in mouse Th17 cells by targeting dCas9-KRAB to a subset of candidate regulatory elements during differentiation. We used FACS to enrich for cells containing gRNA that cause altered expression of the Th17 defining genes *Rorc* and *Il17a*. We combine a transgenic T cell transfer model with CRISPRi to confirm the essentiality of individual regulatory elements for Th17 differentiation *in vivo.* Together these results identify a set of functional regulatory elements that may offer an expanded understanding of the active regulome that governs an adaptive immune response.

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. Among the 4 projects that have been selected for reference epigenome mapping at the McGill EMC platform, 3 are completed.

Aside from the community samples program, McGill EMC works closely with collaborators on various projects by providing technical and analytical expertise. The ongoing implementation of Cut&Run and Cut&Tag techniques as an alternative to ChIP-Seq will allow 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 in order to serve the Canadian epigenetic community.

AGE, SEX, AND CELL-TYPE RESOLVED HYPOTHALAMIC GENE EXPRESSION ACROSS PUBERTY

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Puberty, the period where adults sexually mature to produce gametes and generate secondary sexual characteristics, is a fundamental aspect of development. While the major hormonal aspects of puberty are well understood, genome-wide approaches show that our understanding of the genes, cell-types, and epigenetic mechanisms underlying pubertal regulation is limited. Hypothalamic puberty is challenging when studying it at a genome-wide level because puberty is a continuous, sex-biased, and cellularly heterogeneous process.

To begin to address these challenges we used an automated 3'UTR-seq profiling approach to ascertain gene expression in male and female mice at five timepoints spanning the pubertal transition (N=4-5 per age/timepoint). To characterize the cell-type specific underpinnings of our bulk RNA-seq data, we leveraged public hypothalamic scRNA-seq data with a pre-pubertal and post-pubertal timepoint in mice. Using pairwise differential analysis across age and sex we found the most dynamic hypothalamus gene expression changes before puberty (day 12 to day 22). Overall, the hypothalamus was more dynamic across sex than age. To better capture sex by age interactions, we applied a varimax rotation to a principal component (PC) analysis. This analysis revealed new and novel genes associated with an associated with an age-by-sex interaction across pubertal development. We then used single-cell mapper (scMappR) tool which integrates bulk RNA-seq with single cell RNA-seq. We discovered that many novel cell-type specific genes associated with this age-by-sex interaction played a role in oligodendrocyte expansion. We then leveraged RNA-seq deconvolution and scRNA-seq trajectory analysis of public hypothalamic scRNAseq data to profile oligodendrocyte expansion in the hypothalamus for the first time. Finally, we associated these genes to sex-biased genes in the pituitary gland across puberty and discovered new potential puberty-relevant interactions between the hypothalamus and pituitary. Overall, our findings contribute to our understanding of how puberty influences and is influenced by cellular maturation, neuropeptide activation, and cross-tissue interactions within the hypothalamus.

GATA4/5/6 FAMILY TRANSCRIPTION FACTORS ARE CONSERVED DETERMINANTS OF CARDIAC VERSUS PHARYNGEAL MESODERM FATE

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GATA4/5/6 transcription factors play essential, conserved roles in heart development. How these factors mediate the transition from multipotent mesoderm progenitors to a committed cardiac fate is unclear. To understand how GATA4/5/6 modulate cell fate decisions, we labelled, isolated, and performed single-cell gene expression analysis on cells that express gata5 at pre-cardiac time points spanning gastrulation to somitogenesis. We found that most mesendoderm-derived lineages had dynamic gata5/6 expression. In the absence of Gata5/6, the population structure of mesendoderm-derived cells was dramatically altered. In addition to the expected absence of cardiac mesoderm, we observed a concomitant expansion of cranial-pharyngeal mesoderm. Functional genetic analyses in zebrafish and the invertebrate chordate Ciona, which possess a single GATA4/5/6 homolog, revealed an essential and cell-autonomous role for GATA4/5/6 in promoting cardiac and inhibiting pharyngeal mesoderm identity. Consistent with the known pioneering role of the GATA factors, prior to the establishment of a cardiac fate, Gata5/6 loss led to an extensive loss of accessible chromatin regions (n = 2225) that were enriched for GATA motifs. Supporting a potential repressive role of Gata5/6, their knockdown also led to a gain of accessible chromatin regions (n= 372). Notably, we identified a gain of accessibility of a pharyngeal-specific enhancer near the pharyngeal muscle transcription factor, tbx1. As enhanced tbx1 expression is a conserved feature of both zebrafish and Ciona pharyngeal mesoderm, we propose that balancing the cardiac and pharyngeal mesoderm fates is controlled by a deeply conserved GATA4/5/6-TBX1 regulatory logic.

PRMT7 ABLATION STIMULATES ANTI-TUMOR IMMUNITY AND SENSITIZES MELANOMA TO IMMUNE CHECKPOINT BLOCKADE

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Despite the success of immune checkpoint inhibitor (ICI) therapy in different cancers, resistance and relapses are frequent. Thus, combination therapies are expected to enhance response rates and overcome resistance to ICIs. Herein, we report that combining protein arginine methyltransferase 7 (PRMT7) inhibition with ICIs triggers a strong anti-tumor T cell immunity and restrains tumor growth *in vivo* by increasing tumor immune cell infiltration. Consistently, TCGA database analysis showed an inverse correlation between PRMT7 expression and T cell infiltration in human melanomas. Mechanistically, we show that PRMT7 has a two-prong effect on melanoma tumor immunity. On one hand, it serves as a coactivator of IRF-1 for PD-*L1* expression by upregulating promoter H4R3me2s levels in melanoma cells. Next, PRMT7 prevents repetitive element expression to avoid intracellular dsRNA accumulation or 'viral mimicry'. PRMT7 deletion resulted in increased endogenous retroviral elements (ERVs), dsRNA, and genes implicated in interferon activation, antigen presentation and chemokine signaling. Our findings identify PRMT7 as factor used by melanoma to evade anti-tumor immunity and define the therapeutic potential of PRMT7 alone or in combination with PD-(L)1 blockade to enhance ICI efficiency.

THE HISTONE CHAPERONE ANP32E REGULATES MEMORY FORMATION, TRANSCRIPTION, AND DENDRITIC

MORPHOLOGY BY REGULATING STEADY-STATE H2A.Z BINDING IN NEURONS

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Rapid removal of histone H2A.Z from neuronal chromatin is a key step in learning-induced gene expression and memory formation, but mechanisms underlying learning-induced H2A.Z removal are unclear. Anp32e was recently identified as an H2A.Z-specific histone chaperone that removes H2A.Z from nucleosomes in dividing cells, but its role in non-dividing neurons is unclear. Moreover, prior studies investigated Anp32e function under steady-state rather than stimulus-induced conditions. Here, we show that Anp32e regulates H2A.Z binding in neurons under steady-state conditions, with lesser impact on stimulus-induced H2A.Z removal. Functionally, Anp32e depletion leads to H2A.Z-dependent impairment in transcription and dendritic arborization in cultured hippocampal neurons, as well as impaired recall of contextual fear memory and transcriptional regulation. Together, these data indicate that Anp32e regulates behavioral and morphological outcomes by preventing H2A.Z accumulation in chromatin rather than by regulating activity-mediated H2A.Z dynamics.

HEMATOPOIETIC STEM/PROGENITOR CELL CLASSIFIER BASED ON CHROMATIN ACCESSIBILITY

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Background: Examining blood cell differentiation *in vivo* is a key component of studying blood disorders such as leukemia. Cells must undergo epigenetic changes in order to modify their gene expression and follow different cell differentiation trajectories. Here, we postulate that chromatin accessibility is sufficiently dissimilar between different subsets of hematopoietic stem/progenitor cells to determine cell lineage.

Methods: 1343 sequenced scATAC-seq libraries generated from flow-sorted normal CD34+ human bone marrow were used. Nine distinct cell types were identified by flow markers, and HSC and MPP classes were combined resulting in 8 unique targets. Z-score-adjusted chromatin accessibility features were based on imputed transcription factor (TF) activity using chromVAR. To avoid class imbalance, over-represented cell types were down-sampled to 120 observations each, retaining 1005 libraries. A gradient-boosted decision tree model was trained on the TF accessibility of 870 TFs for 669 observations to classify single-cell libraries into blood cell types. Performance was evaluated using 5-fold cross-validation on the test set (n = 336).

Results: Prior to the combination of HSC and MPP classes, a moderate degree of confusion was observed between the two suggesting that they have similar chromatin accessibility and TF activity. After joining the classes, we observed high prediction accuracy for the remaining 8 targets, with receiver-operator curve (ROC) area under the curve (AUC) values ranging from 0.96 to 1.00. The weighted average precision was 0.840 and the weighted average recall was 0.818, for an overall F1-score of 0.822. Feature importance analyses identified TFs EBF1, BCL11A, GATA3, and SPIB as major differentiators of cell lineage which is consistent with the literature.

Conclusions: Based on our findings, it is possible to confidently determine the lineage of a cell based solely on single-cell chromatin accessibility features. Using this classifier, large-scale scATAC-seq approaches without cell surface marker information can be labelled with likely cell types. There is a broad application for this tool in the study of many various blood cell disorders. While this work suggests that TF accessibility is sufficiently different between the various cell types, the features used were limited based on summarization on known TF footprints. Novel genomic regions of interest may be discovered from training a similar classifier on raw chromatin accessibility.

A NEAR-NATIVE SYSTEM FOR BIOCHEMICAL ANALYSIS OF NUCLEAR-LOCALIZED METABOLIC ENZYMES THAT PRODUCE EPIGENETIC METABOLITES.

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Nuclear-localized metabolic enzymes are thought to contribute to gene regulation by modulating the availability of metabolites used by writers and erasers of epigenetic marks. Deep understanding of this regulatory axis will benefit from knowledge of the kinetic properties of nuclear enzymes; obtaining this knowledge for enzymes in their native state and native environment will however be challenging[1]. To meet this challenge, we are developing top-down methods that take advantage of several unique features of the *Xenopus laevis* oocyte as a cell model for biochemical analysis of enzyme activity.

Whole nuclei and whole cytoplasms are prepared in their native state by dissecting oocytes under a biocompatible oil. Nuclei and cytoplasms are homogenized in a near-native buffer for whole compartment analysis of enzyme kinetics. We focus on enzymes that produce or consume NAD(P)H because these metabolites are readily detected by non-invasive spectrophotometry. The volumes of these compartments (our data) and published absolute concentrations of enzymes in the oocyte nucleus and cytoplasm[2] are used to estimate enzyme amounts assayed.

Here we address a straightforward question for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a classical glycolytic enzyme also found in the nucleus: does the GAPDH population of the whole nuclear compartment have the same kinetic profile as the GAPDH population of the whole cytoplasm?

As expected, cytoplasmic GAPDH is a robust catalyst of the glyceraldehyde 3-phosphate (G3P) + NAD^+ > glycerol 1,3-bisphosphate + NADH reaction. The same is true for the whole GAPDH population of homogenized native nuclei. These enzyme populations however differ in some classical kinetic properties, specifically apparent V_{max} and substrate inhibition by G3P. The implication of this finding is that flux through nuclear GAPDH is not controlled in the same way as flux though cytoplasmic GAPDH. It follows that metabolic enzymology relevant to epigenetic regulation may not be fully revealed by studying purified GAPDH or cytoplasmic GAPDH.

This finding sets the stage for mechanistic analysis of the control of the nuclear and cytoplasmic populations of GAPDH. Potential mechanisms of interest include post-transcriptional modification of GAPDH and nucleocytoplasmic compartmentation of allosteric regulators (for example, metabolites and RNA).

1. Schvartzman JM et al. Metabolic regulation of chromatin modifications and gene expression. J Cell Biol. 2018 217:2247. 2. Kırlı K et al. A deep proteomics perspective on CRM1-mediated nuclear export and nucleocytoplasmic partitioning. eLife. 2015 4:e11466.
2. Supported by a CIHR project grant.

MATERNAL BODY MASS INDEX IS ASSOCIATED WITH PLASMATIC MICRORNAS PROFILE VARIATIONS AT FIRST TRIMESTER OF PREGNANCY

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Background: Overweight and obesity (OWO) affect nearly two thirds of the world's population, including pregnant women. Maternal OWO is a strong risk factor for several pregnancy complications among which preeclampsia and gestational diabetes are the most common. These complications are associated with short and long term health consequences for both the mother and her child. Epigenetic mechanisms, including microRNAs (miRNAs), play an important role in the interaction between genes and the environment. MiRNAs are short RNA molecules (19 to 24 nucleotides) secreted and stable in blood. They are known to regulate several biological processes, some of which linked to obesity and diabetes, such as glucose and lipids metabolism, insulin resistance and chronic inflammation. Very few studies have assessed the association between the plasma miRNA profile and the presence of overweight and obesity in pregnancy. **Hypothesis**: Maternal body mass index (BMI) is associated with plasmatic miRNA profile variations at first trimester of pregnancy. **Objective**: To identify plasmatic miRNAs, measured in the first trimester, associated with maternal BMI at first trimester of pregnancy and metabolic pathways they are regulating. **Methods**: Total RNA species were extracted from plasma samples collected between the 6th and 16th week of pregnancy and from 435 and 223 pregnant women participating to the Gen3G (discovery) and 3D (replication) prospective birth cohorts. MiRNAs were quantified using next-generation sequencing. Then, the DESeq2 package was applied to identify miRNAs associated with maternal BMI measured in the 1st trimester of pregnancy. BMI was analyzed as a continuous variable. Analyses were adjusted for sequencing runs and lanes, maternal age, gestational age at the time of blood collection and parity. Finally, KEGG metabolic pathway analyses were performed using mirPath v.3 software. **Results**: In Gen3G, we identify 61 miRNAs associated with maternal BMI ($q<0.05$). Of these, 32 miRNAs are located within genomic clusters known to be more specifically expressed in the placenta: chromosome 19 (28 miRNAs), chromosome 14 (1 miRNA) and miR-371-3 (3 miRNAs) miRNA clusters. These miRNAs are involved in biological pathways related to obesity such as the synthesis, metabolism and elongation of fatty acids, among others. Finally, 38 out of 61 miRNAs were replicated in the 3D cohort based on the direction and the strength of the associations (fold change): 17 passed the significance threshold ($p<0.05$). **Conclusion**: We have identified 61 miRNAs associated with maternal BMI in the 1st trimester of pregnancy, of which 17 were replicated in an independent cohort. These miRNAs are enriched in biological pathways linked to fatty acid metabolism and some of them are found in placenta associated miRNAs clusters. These miRNAs could mediate the association between maternal OWO and pregnancy complications.

RIF1 IMPAIRS DNA REPLICATION IN ABSENCE OF SIRTUINS-MEDIATED HISTONE DEACETYLATION

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Chromatin structure influences the ability of cells to respond to impediments to DNA replication fork progression, i.e., replicative stress. In the yeast *Saccharomyces cerevisiae*, newly synthesized histone H3 is acetylated on lysine 56 (H3K56ac),

then deposited behind DNA replication forks. H3K56ac is subsequently deacetylated upon completion of S phase by two members of the class III histone deacetylase (sirtuin) family, Hst3 and Hst4, except in the presence of replication stress, in which case H3K56ac persists. It has been reported that cells lacking Hst3 and Hst4 present constitutive H3K56ac, which activates S phase checkpoint, leads to elevated recombinational foci and extreme sensitivity to replicative stress. However, the molecular mechanisms linking DNA replication stress with nascent chromatin structure remain incompletely characterized.

We performed a genome-wide screen of the heterozygous yeast gene deletion collection to identify essential genes that promote cell fitness upon inhibition of sirtuins by nicotinamide (NAM). Heterozygosity of genes promoting DNA replication initiation caused NAM sensitivity in a H3K56ac-dependent manner. Consistently, we found that reducing origin activity in an *hst3Δ hst4Δ* stain cause very slow S phase progression. Interestingly, deletion of the gene encoding Rap1-interacting factor 1 (Rif1), which in turn elevates activity of replication's origins genome-wide, suppressed NAM-induced S phase progression defects, along with the temperature sensitivity of *hst3Δ hst4Δ* mutants. This effect is independent of the telomere homeostasis function of Rif1. We further determined that recombinational foci observed in cells with constitutive acetylation of H3K56 depends on Rif1 activity. Surprisingly, suppression of the S phase checkpoint does not restore S phase progression of an *hst3Δ hst4Δ* mutants with low origin activity, suggesting that acetylation of H3K56 might direct modulate replication origin activity.

Altogether, our results suggest that abnormal nascent chromatin structure negatively regulates DNA replication origin activity, thereby causing Rif1-dependent DNA damage.

FREQUENTLY MUTATED TOP2B BINDING SITES REVEAL CANDIDATE STRUCTURAL AND REGULATORY CANCER DRIVES OF HEPATOCELLULAR CARCINOMA

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Whole genome sequencing (WGS) of many cancer types has revealed an enrichment of somatic mutations at the binding sites of CTCF and the cohesin complex at the topologically associating domain (TAD) boundaries. This is an unexplained phenomenon that is especially evident in hepatocellular carcinoma (HCC). We previously demonstrated that Topoisomerase II beta (TOP2B), an enzyme that catalyses transient DNA double strand breaks (DSBs), interacts and extensively co-localizes with CTCF and cohesin in mouse liver, leading to the hypothesis that TOP2B activities may contribute to the mutational processes in HCC.

To understand TOP2B's role in somatic mutagenesis in HCC, we characterized the genome-wide binding of TOP2B, CTCF and the cohesin complex subunit RAD21 in primary human HCC tumors. Our analysis of somatic mutations in the WGS data of 300 HCC tumors demonstrated that the enrichment of somatic mutations is focal to the co-binding of TOP2B, CTCF and RAD21, including TAD boundary regions shared by a large number of human tissues. Interestingly, TOP2B-RAD21 binding sites associated with liver enhancers are also strongly enriched in mutations. Genome-wide cancer driver discovery using our ActiveDriverWGS method revealed 49 frequently mutated candidate drivers in HCC, 36 of which occurred at TOP2B binding sites. Furthermore, we observed significant non-coding driver mutations in additional cancer types that occur in tissues subjected to exogenous agents (e.g. esophageous, stomach). Since the majority of candidate drivers are located in TOP2B-associated, non-coding structural and regulatory elements of the liver genome, TOP2B-mediated DSBs may thus represent a mutational mechanism of underlying HCC.

EPIGENETIC AGE PREDICTION IN A LARGE-SCALE METHYLATION SEQUENCING PROJECT

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The Horvath epigenetic clock is a biomarker of aging that includes 353 age-informative CpG sites and leverages methylation changes over time at these sites to predict epigenetic age. The Horvath algorithm is the most frequently used epigenetic clock and has proven accuracy in array-based studies and adult samples with a median absolute error (AE; AE= abs(predicted age (PA)- chronological age (number of calendar years since birth, CA)) of 3.6 years in an independent validation set.

There are three main **objectives** to this study: 1) Assess the accuracy of the Horvath clock in targeted methylation sequencing data, 2) Evaluate its applicability and utility as a quality control (QC) metric, 3) Identify novel age-informative CpG sites in the Illumina TruSeq Methyl Capture library.

The study population (Figure 1) includes 812 samples from The Canadian Asthma Primary Prevention Study (CAPPS, n=632) and the Saguenay-Lac-Saint-Jean study (SLSJ, n=180

samples). CAPPS is a longitudinal birth-cohort which follows children at high risk of developing asthma from birth to age 15 and includes methylation sequencing at one or multiple time points. SLSJ consists of three-generational triads of French-Canadian families. An independent set of 120 samples of predominantly pediatric peanut allergy cases from the Canadian Peanut Allergy Registry (CanPAR) was sequenced separately.

Sequencing was completed at the Genome Sciences Center in Vancouver, BC for both sequencing experiments. QC metrics including PCR sex check, Principal Component Analysis (PCA on ethnicity, age, cell composition), genotype concordance and identity by state (IBS) were applied on the CAPPS and SLSJ samples with 795 of the 812 remaining in the study. Linear regression and mixed effects analysis were performed on these samples to identify age-informative CpG sites with a transformed version of age as the dependent variable and methylation as the independent variable.

Results: The mean AE between the Horvath-PA and the CA was ± 6.77 years (SD:7.03, median=4.17) for the CAPPS and SLSJ samples which passed QC and ± 8.55 years (SD:6.89, median=7.00) for the CanPAR samples. The Horvath clock, in combination with independent QC metrics, accurately identified swapped and mislabelled samples. Thousands of novel CpG sites significantly associated with age were identified using linear regression (582,187 CpG with p-value < $1x10^{-8}$) and mixed effects modelling (559,870 with p-value < $1x10^{-8}$).

Conclusions: We have demonstrated that the Horvath epigenetic clock is accurate in targeted methylation sequencing data and has utility as a QC metric. The higher median AE (CAPPS and SLSJ=4.17 and CanPAR =7.0) observed in our samples vs. Horvath's original study (median AE=3.6), and the identification of novel CpG sites associated with age indicates an opportunity to develop a novel and more precise epigenetic clock that will aid in the investigation of epigenetic mechanism of disease phenotypes.

EZH2 INHIBITION ALTERS THE LINEAGE OUTPUTS OF HEMATOPOIETIC PROGENITORS WITH B LYMPHOID AND NEUTROPHIL/MONOCYTE RESTRICTED ACTIVITY

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Human B lymphocytes (B) and neutrophils/monocytes (NM) are now known to be generated from a common progenitor restricted to these lineage outputs. But the molecular changes and intrinsic events that determine this process remain poorly understood, in particular the nature and role(s) of specific changes in the epigenome that take place. To address this question, we first performed histone modification profiling of FACS-purified subsets of normal human cord blood (CB) CD34+ cells that are differentially enriched for different types of mature lineage cells and their various progenitors. The results showed that circulating neonatal monocytes and erythroblasts show a genome-wide contraction of H3K27me3 density compared to the CD34+ progenitors. In contrast, co-isolated B and T cells displayed a H3K27me3 profile more similar to the progenitor cells. To test the hypothesis that this difference in H3K27me3 modification is part of the B/NM lineage restriction process, we investigated the effect of an EZH2 inhibitor (EPZ-6438) on NM and B outputs in an optimized culture system that supports lympho-myeloid progenitor differentiation. The results showed the presence of EPZ-6438 caused a selective decrease in CD19+ B-lineage cells following 14-days in culture. To determine if this result reflects an early step in the lineage restriction process, we compared the 6-day outputs of the unrestricted CD34+CD45RA-CLEC12A-(RA-C-) CB population in cultures with and without added EPZ-6438. This revealed a significant and selective inhibition of CD45RA+CLEC12A-(RA+C-) B lineage-restricted cell outputs, whereas the production of NM lineage cells was concomitantly increased. Clonal analysis of this effect further showed that the percentage of progenitors able to produce both NM and B lineages was reduced by EPZ-6438 treatment, whereas the frequency of clonogenic cells with NM-only lineage output was increased. Together, these results suggest the global reduction of H3K27me levels by inhibiting EZH2 initiates a loss of B lineage potential by NM+B progenitors and an associated promotion of a NM lineage program. Taken together, these findings suggest a key role for H3K27me3 in the regulation of final events of human NM+B lineage restriction.

EFFECTS OF PATERNAL HIGH-FAT DIET AND MATERNAL REARING ENVIRONMENT ON THE MICROBIOTA-GUT-BRAIN AXIS AND BEHAVIOR

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Exposing a male rat to an obesogenic high-fat diet (HFD) influences attractiveness to potential female mates, the subsequent interaction of female mates with infant offspring, and the development of stress-related behavioral and neural responses in offspring. To examine the stomach and fecal microbiome's potential roles, fecal samples from 44 offspring and stomach samples from offspring and their fathers were collected and bacterial community composition was studied by 16 small subunit ribosomal RNA (16S rRNA) gene sequencing. Paternal diet (control, high-fat), maternal housing conditions (standard or semi-naturalistic housing), and maternal care

(quality of nursing and other maternal behaviors) affected the within-subjects alpha-diversity of the offspring stomach and fecal microbiomes. We provide evidence from beta-diversity analyses that paternal diet and maternal behavior induce community-wide shifts to the adult offspring gut microbiome. Additionally, we show that paternal HFD significantly alters the adult offspring Firmicutes to Bacteroides ratio, an indicator of obesogenic potential in the gut microbiome. Additional machine-learning analyses indicate that microbial species driving these differences converge on *Bifidobacterium pseudolongum.* These results suggest that differences in early-life care induced by paternal diet and maternal care significantly influence the microbiota composition of offspring through the microbiota-gut-brain axis, having implications for adult stress reactivity.

REMOVAL OF EPIGENETIC BARRIER ENABLES ESCS TO ACCESS TROPHOBLAST AND PRIMITIVE ENDODERM FATES AND SELF-ASSEMBLE INTO BLASTOFFS

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A blastoid model generated from a single cell can provide clues into the Blackbox of early lineage specification. Current murine models well illustrate lineage self- assembly but circumvent fate specification by combining fully differentiated trophectoderm (TE) and embryonic (EPI) lineages. The objective is to reprogram ESCs to a ”plastic” state to enable access to restricted trophectoderm (TE) and primitive endoderm (PrE) lineages. Closer replication of in vivo development may provide greater opportunity to study the first essential lineage choices in development. I use epigenetic reprogramming, via specific histone deacetylase (HDAC) 1-3 chemical inhibition of ESCs, to promote a permissive and accessible chromatin state. HDAC-inhibited ESCs grown in non-adherent cultures aggregated in blastocyst-like structures with spatial organization. Results of transcript (rt-qPCR) and protein expression (immune-fluorescence) analysis confirm that HDAC1-3 inhibited ESCs can access the restricted trophoblast fate via a “plastic” intermediary. Significant increase in expanded potential marker Zscan4, is seen after 3 days of HDAC inhibition. After a longer HDAC inhibition duration (6 days), trophoblastic (Cdx2 & Eomes) and PrE lineage (Sox17) markers significantly increase during aggregate culture. Optimal culture media produced a high percentage of blastocyst-like structures (>10%). In summary, I demonstrate evidence that HDAC1-3 inhibited ESCs have the capacity to access alternative TE and PrE fates and self-assemble into blastocyst-like structures.

UNTANGLING THE IMPACT OF CFP1 MUTATIONS IN EPIGENETIC SIGNALING

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Histone H3 lysine 4 trimethylation (H3K4me3) is an evolutionarily conserved post-translational modification predominantly found at promoter sequences in transcriptionally active euchromatin. In humans, the most abundant enzyme that catalyzes this mark is the Set1 methyltransferase complex. This complex is made up of multiple subunits that are important in stimulating the catalytic activity of Set1 and subsequent recruitment of RNA polymerase II. One of the regulatory subunits in this complex is CFP1, which interacts with SET1, acts as a tether for the complex to dock on unmethylated CpG islands, and recognizes H3K4me3 marks with its conserved PHD domain. In cancer patients, various mutations in CFP1 have been identified, however little is known about how these mutations affect the epigenome or gene expression. We sought to identify the impact of 6 mutations altering conserved residues in the PHD domain. We hypothesize that these mutations significantly impact CFP1’s epigenetic regulation by reducing its stability and/or reducing its ability to recognize H3K4 methylation marks. To study this, we measured the relative stability of mutants via Western blot and measured the relative binding ability via pulldown assays. We found that the H51Y mutant greatly reduced CFP1 stability *in vitro*, and all of our mutations ablated H3K4me3 binding except K63N and R74T. With a better understanding of how CFP1 mutations affect function, we can paint a better picture of how CFP1 plays into the complex etiology of numerous cancers.

INVERSE-COVARIANCE REGULARIZED SPARSE MULTIVARIATE REGRESSION FOR IDENTIFYING METHYLATION QUANTITATIVE TRAIT LOCI WITH MISSING DATA

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Introduction: DNA methylation are locally correlated, and often strongly influenced by proximal SNPs. Such associations can be modeled with multivariate (methylation at nearby CpGs) regressions with input variables (SNPs) lying in a high-dimensional space. In the standard formulation of this problem, the outputs are assumed to be fully observed. However, this assumption is violated in many applications. For example, in high-throughput sequencing, it is common for measurements to be missing at some positions in some samples due to stochasticity in the sequencing capture. Although there are many existing techniques for dealing with missing data, they can be challenging to implement because of non-convexity in optimizations. We introduce an efficient method for inverse-

covariance regularized sparse multivariate regression when outputs contain missing values, motivated by methylation data.

Methods: Building on error-in-variables concepts, the intermediate variables in optimization procedures are replaced with designed unbiased surrogates. This approach iterates over two convex sub-problems and achieves an improvement in computational efficiency compared to non-convex techniques.

Results: The method is implemented in Rcpp. In simulations, while varying missing rate and sample size, we evaluate performance measured by whether it recovers the structured sparsity in regression coefficients and in the network structure among the outputs. Preliminary results indicate that our method shows high efficiency and robustness across different scenarios. We apply our method to a corrupted DNA methylation dataset, to identify the subset of SNPs that contribute to region-wise methylation differences, and also the conditional network structures among the CpGs in regions after correcting for genetic effects.

Discussion: Additional types of data corruption such as additive error, may be considered in future work.

SEX CHROMOSOMES AND SEX PHENOTYPE CONTRIBUTE TO BIASED DNA METHYLATION IN MOUSE LIVER AND BRAIN

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Mammalian males and females differ from multiple perspectives spanning DNA methylome and transcriptome, as well as levels of sex hormones, physiology, and risks of developing diseases. Historically, researchers focused more on gonadal sex hormones but underexplored epigenetic contributions that are sex hormone-independent. We have managed to generate sex-reversed mice, which provides precious opportunities to capture epigenomic patterns associated with phenotypic sex and sex chromosome complement separately, contributing to a better understanding of sex-associated DNA methylome under such a new framework. Specifically, samples include liver and whole-brain tissues from mice of XX females (XX.F), XY males (XY.M), and XY females (XY.F). We also detected enrichment of sex-associated differentially methylated region (**sDMR**) of CpG and CAC among regulatory regions, such as promoters and enhancers, with more sDMRs and differentially expressed genes identified in liver samples. In liver samples, a majority of autosomal CpG sDMRs showed higher methylation levels in XX.F and XY.F than XY.M, in contrast, and only limited autosomal sDMRs were identified between XX.F and XY.F, whereas brain samples showed higher percentages of autosomal sDMRs with higher methylations in XY.F and XY.M compared to XX.F. In both liver and brain samples, X-linked sDMRs showed higher methylation levels in XX.F than XY.F and XY.M as expected. However, X-linked CAC sDMRs in the brain showed a reverse trend as CpG sDMRs (lower methylation levels in XX.F than XY.F and XY.M). Furthermore, we find enrichment of sDMR in the vicinity of differentially expressed genes which shows promises of utilizing sDMR as probes to explore potential regulatory mechanisms between DNA methylome and transcriptome.

MUTATIONAL SIGNATURES OF ALTERNATIVE DNA REPAIR PATHWAYS ASSOCIATED WITH TRANSCRIPTION IN HUMAN CANCER CELLS

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Genomic instability is a defining characteristic of cancer development. High throughput genome sequencing of large amounts of human cancer genomes and normal tissues allows the identification of mutations that appear during cancer development (somatic mutations). Understanding the mutational processes involved in the formation of somatic mutations such as single base substitutions (SBS), small insertions and deletions (indels) and more complex rearrangements can lead to better understanding of DNA damage response (DDR) pathways and cancer vulnerabilities. Defects in DDR are often the basis of the accumulation of somatic mutations in cancer cells and can leave specific patterns of somatic mutations (mutational signatures). Illudin S (ILS), a cytotoxic secondary metabolite, is known to stall DNA replication forks and generate replication stress, a key factor in genomic instability. Cancer cells with high levels of replication stress have shown a higher sensitivity to specific treatments. In this study we implemented an experimental and analytical protocol to identify the mutational signature of replication stress. By exposing HAP1 cell lines to inhibitory concentrations of ILS, we found a novel SBS mutational signature. This signature is altered when the treatment is combined with CRISPR knockdowns of certain DDR genes, suggesting the involvement of error prone DDR pathways in the repair of ILS induced damage. We observed a transcription strand bias which might reveal a role of Transcription Coupled (TC) repair pathways in the response to ILS damage. To validate this hypothesis, we associated gene expression (RNA-seq) and DNA accessibility data with ILS induced mutations in cancer cell line genomes.

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