Canadian Conference in Organoid Research

Conférence Canadienne sur la Rechereche Organoïde

Program & Abstracts

Nov 5 – 6, 2025
London, ON, Canada

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Land Acknowledgement:

We acknowledge that we are gathered in the city of London, Ontario which is located on the traditional lands of the Anishinaabek, Haudenosaunee, Lūnaapéewak and Chonnonton Nations, on lands connected with the London Township and Sombra Treaties of 1796 and the Dish with One Spoon Covenant Wampum.

This land continues to be home to diverse Indigenous Peoples (First Nations, Métis, and Inuit) whom we recognize as contemporary stewards of the land and vital contributors of our society. Where possible, we seek to renew respectful relationships with Indigenous communities through our teaching, research and community service.

Welcome / Bienvenue

We are thrilled to welcome you to the first Canadian Conference in Organoid Research (CANCOR) – Conférence Canadienne sur la Recherche Organoïde (CCRO).

Organoid models have numerous applications in fundamental and biomedical research including disease modeling, drug testing and development, personalized medicine, regenerative medicine, and infectious disease research. We are pleased our conference program includes researchers from across Canada who are at the forefront of developing and incorporating organoid models into their research program. We have also highlighted the work of trainees and early-career researchers who represent the next-generation of innovators in the field. Finally, we are excited to have a pioneer in the organoid field, Dr. Hans Clevers a Professor of Molecular Genetics from Utrecht University, present our keynote talk.

With this conference, our goal is to bring together Canadian clinical and basic scientists for the first time to share their organoid research. We hope the engaged discussions from this event will cultivate new interdisciplinary and translational collaborations to ultimately advance the application of organoids to model human disease and personalize screening of therapeutics.

We look forward to an amazing conference!

Sincerely,

The CANCOR-CCRO Organizing Committee

Christopher Pin
Professor
Western University

Verspeeten Family Cancer Centre
London Health Science Research Institute

Trevor Shepherd
Associate Professor
Western University

The Mary & John Knight Translational Ovarian Cancer Research Unit
London Regional Cancer Program

Van Lu
Assistant Professor
Western University

Dean Betts
Professor
Western University

Division Chair
Children's Health Research Institute

Samuel Asfaha
Associate Professor
Western University

Verspeeten Family Cancer Centre
London Health Science Research Institute

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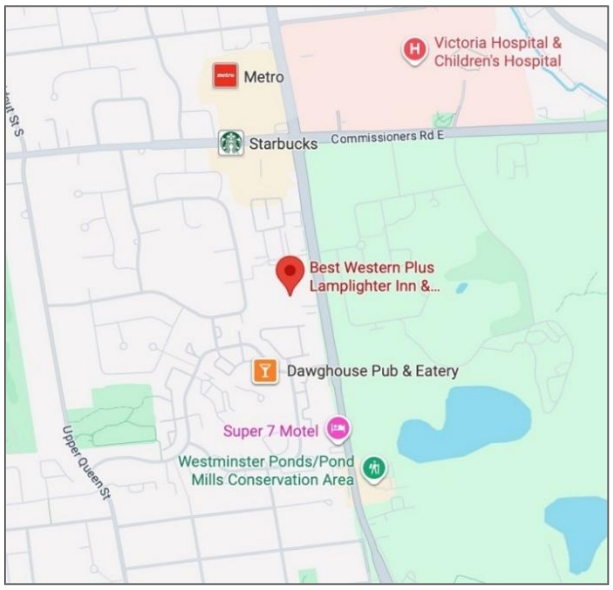
Venue Location

CANCOR-CCRO will be held at the Best Western Plus Lamplighter Inn & Conference Centre in London, Ontario, Canada.



Best Western Plus Lamplighter Inn & Conference Centre

591 Wellington Road, London, ON N6C 4R3

Phone: 519-681-7151

For attendees staying at the Lamplighter Inn, **check in** time is **4:00 PM** on the day of your stay and **check out** is **11:00 AM.**

If you have any question, please do not hesitate to reach out to our team. We want to ensure you enjoy your time visiting.

Driving Directions

Attendees **driving** to the event, coming off **401 West (Toronto)**, take exit 187 for Exeter Road. Then turn right onto Wellington Road and continue straight until you reach the Lamplighter on your left.

Attendees coming off **401 East (Windsor)**, turn north onto Wellington Road and continue straight until you reach the Lamplighter on your left. Please be aware that there is currently on-going construction on Wellington Road.

Complimentary parking available to all guests.

Looking for places to eat?

Here is a complied list of restaurants arranged by proximity to the Lamplighter Inn.

- Shelly's Tap and Grill, on the premises of the Lamplighter Inn. Open for breakfast, lunch, dinner (from 7 AM to 9 PM) Located off the lobby with access from the north parking lot. Phone number: 519-691-3402; Lounge: 519-691-3428.
- The Mandarin is a buffet style Chinese restaurant and is open from 11:30 AM to 9:00 PM for lunch and dinner. Directions available here. Phone number: 519-680-5000.
- Crabby Joe's Bar and Grill is an American restaurant open from 11:00 AM to 12:00 AM for lunch and dinner. Directions available here. Phone number: 519-686-7888.
- Domino's Pizza is a fast-food restaurant open from 10:30 AM to 12:00 PM for lunch, and dinner. Directions available here.
- McDonald's is a fast-food restaurant open from 6:00 AM to 10:00 PM for breakfast, lunch and dinner. Directions available here.
- Tim Horton's is a fast-food restaurant open 24 hours. Directions available here.
- Starbucks is a coffee shop open from 6:00 AM to 9:00 PM for breakfast, lunch and dinner. Directions available here.
- The Keg is a steak house restaurant open from 4:00 PM to 11:00 PM for dinner. Directions available here. Phone number: 519-686-5811.

Conference Centre

The Conference Centre is located past the hotel entrance. Ample parking and the accessible entrance can be found on the south side of the Conference Centre.

Parking is available at the conference centre free of charge.

Registration desk is located outside the Crystal Ballroom.

Sessions and Poster Presentations will be held in the Crystal Ballroom.

Meals and Networking Event will be held in the Regency Ballroom.

Agenda

Wednesday November 5, 2025; 7:30 AM – 5:45 PM

Lamplighter Inn & Conference Centre, London, Ontario

7:30 – 8:30 AM	**Registration (Atrium) and Breakfast**	**Regency Ballroom**

8:30 – 8:45 AM	**Introductions and Meeting kick off**	**Crystal Ballroom**

Session 1: Organoids in Gastrointestinal Development and Disease **Moderators:** Samuel Asfaha and Frederikke Larsen (Western University)		
8:45 – 9:10 AM	Van Lu, Assistant Professor, Western University *"Generating novel human intestinal organoid models to investigate human-specific enteroendocrine cell populations"*	
9:10 – 9:35 AM	Simon Hirota, Professor, University of Calgary *"Bugs, drugs and inflammation - beyond cell lines"*	
9:35 – 9:50 AM	Katherine Walton, Assistant Professor, University of Michigan *"A novel model for inducing damage in human intestinal organoids in vivo using imaging to guide directed irradiation"*	
9:50 – 10:15 AM	Bruce Vallance, Professor, University of British Columbia *"Modelling microbe-host interactions at the colonic mucosal surface"*	

10:15 – 10:30 AM	**Coffee Break**	**Regency Ballroom**

Rapid Fire Talks Session I **Moderator:** Trevor Shepard (Western University)	**Crystal Ballroom**
10:30 – 10:45 AM	Britney Tian, University Health Network *"Bioengineered 3D hPSC-cholangiocyte ducts with physiological signals for biliary disease modelling"*
	Chaitali Chitnis, University of Michigan *"Simulating The Physicochemical Influences of Amniotic Fluid in Human Intestinal Organoid Culture"*
	Fatemeh Behjati, Western University *"Interpretable variational autoencoder for integrating epigenetic and transcriptomic profiles in Pancreatic Cancer"*
	Haseeb Mahmood, Western University *"Investigating Prenatal Cannabinoid Exposure and Schizophrenia Vulnerability Via Lipidomic Alternations and Impaired Neurodevelopment"*
	Michelle Sue, University Health Network *"Generation of Patient-Derived Human Liver Organoids from Fresh and Cryopreserved Human Liver Homogenate"*

Keynote Address	**Crystal Ballroom**	
10:45 – 12:00 PM	Hans Clevers, Hubrecht Institute, Institute of Human Biology *"Organoids in 2D and 3D to model human disease"*	

12:00 – 12:30 PM	**Group Photo**	

12:30 – 2:30 PM	**Lunch** **Poster Session I**	**Regency Ballroom** **Crystal Ballroom**

Session 2: Organoids for Cancer Research **Moderators:** Jim Petrik and Leslie Jeffries (University of Guelph)		
2:30 – 2:55 PM	Véronique Giroux, Associate Professor, Université de Sherbrooke *"Building coculture organoid models to study the interaction between cancer stem cells and the tumor microenvironment"*	
2:55 – 3:20 PM	Franco Vizeacoumar, Professor, Saskatchewan Cancer Agency *"Organoid-Based Therapeutic Discovery and Precision Oncology"*	
3:20 – 3:35 PM	Eugenia Flores-Figueroa, Scientific Associate, University Health Network *"Dissecting Morphological Heterogeneity in Pancreatic Cancer Identifies Distinct Basal Cell States via Multi-Omic and PDO Analysis"*	
3:35 – 4:00 PM	François Boudreau, Professor, Université de Sherbrooke *"Modelling Colon Cancer using Patient-Derived Colonoids and Components of the Tumour Microenvironment"*	

4:00 – 4:15 PM	**Coffee Break**	**Regency Ballroom**

Session 3: Technological Advances in Organoid Research **Moderators:** John Ronald and Emily Tomas (Western University)		
4:15 – 4:40 PM	Darcy Wagner, Professor, McGill University *"The importance of the extracellular niche in regulating lung and airway organoid phenotypes"*	
4:40 – 4:55 PM	Fatemeh Ahmadi, Postdoctoral Fellow, University of Toronto *"Millimeter-Scale Tumoroids Recapitulate In Vivo Tumor Architecture and Enhance Prediction of Complex Drug Responses"*	

4:55 – 5:20 PM	Boyang Zhang, Associate Professor, McMaster University *"Scalable and Automated Culture Platforms for Enhanced Organoid and Tissue Modeling of Human Disease"*	
5:20 – 5:45 PM	Jacob Shelley, Associate Professor, Western University *"Navigating the Legal and Ethical Challenges of Personalized Medicine and Organoid Technologies"*	

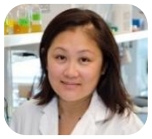
5:45 – 7:00 PM	**Networking Mixer** courtesy of Millipore-Sigma Social Activity: Photo booth	**Regency Ballroom**

End of Day 1

Thursday November 6, 2025; 7:30 AM – 4:30 PM

Lamplighter Inn & Conference Centre, London, Ontario

7:30 – 8:30 AM	**Registration (Atrium) and Breakfast** **Meet the Speakers Networking Event**	**Regency Ballroom**

Session 4: Organoid Development from iPSCs		**Crystal Ballroom**
Moderators: Dean Betts and Karly Masse (Western University)		
8:30 – 8:55 AM	Amy Wong, Scientist, Sick Kids *"Modeling human lung development and disease using induced pluripotent stem cell-derived organoids"*	
8:55 – 9:20 AM	Jessica Esseltine, Associate Professor, Memorial University *"Apically localized PANX1 impacts neuroepithelial expansion in human cerebral organoids"*	
9:20 – 9:35 AM	Ali Mousavi, Postdoctoral Fellow, University of Montréal *"Development of a 3D beating heart-on-a-chip for modeling dilated cardiomyopathy using patient-specific stem cells"*	
9:35 – 10:00 AM	Kalina Kamenova, Research Director, Genomics4S *"Ethical Issues in Organoid Research: Towards a Public Consensus"*	

Panel Discussion: Organoid Resource in Canada		**Moderator:** Christopher Pin
10:00 – 10:10 AM	Nikolina Radulovich, Staff Scientist, University Health Network *"The Princess Margaret Living Biobank: A Resource for Translational Cancer Research"*	
10:10 – 10:20 AM	Hyunjae Chung, Senior Stem Cell Scientist, University of Calgary *"Advancing Organoid Research through Shared Resources and Biobanking"*	
10:20 – 10:40 AM	Panel Discussion with Nikolina Radulovich, Hyunjae Chung, Kalina Kamenova, Jacob Shelley	

10:40 – 10:55 AM	**Coffee Break**	**Regency Ballroom**

Rapid Fire Talks Session II **Moderator:** Trevor Shepard (Western University)	**Crystal Ballroom**
10:55 – 11:10 AM	Abbie Lo, McEwen University Health Network *"Development of hPSC-derived Multi-Lineage Liver Organoids Incorporating Hepatic Stellate Cells for Hepatic Maturation"*
	Frances Sutherland, Western University *"Endothelial Dysfunction in Loeys-Dietz Syndrome: Insights from Patient Tissue and development of a hiPSC-derived Microfluidic Model"*
	Jonathan Villanueva, University of Michigan *"Interrogating Diverse Injury Response Programs in the Damaged Human Intestine Across Different Injuries"*
	Leslie Jeffries, University of Guelph *"Fc3TSR induces cytotoxicity of epithelial ovarian cancer patient-derived organoids"*
	Petra Samardzija, Western University *"A novel method to measure Ca^{2+} entry in patient-derived pancreatic cancer organoids"*

Session 5: Organoids for Studying Chronic Disease **Moderators:** François Boudreau (U Sherbrooke) and Nick Philbin (University of Windsor)		
11:10 – 11:35 AM	Justin Chun, Associate Professor, University of Calgary *"Kidney Organoids in Disease Modelling: Progress and Emerging Opportunities"*	
11:35 – 12:00 PM	Shinichiro Ogawa, Assistant Professor, University Health Network *"Developing Stem Cell-Derived Liver Organoids to Study and Treat Liver Disease"*	
12:00 – 12:15 PM	Darasimi Kola-Ilesanmi, MSc candidate, University of Calgary *"Partial EMT drives collective epithelial cell migration during Crohn's disease fistula formation: A human intestinal organoid model"*	

12:15 – 2:15 PM	**Lunch** **Poster Session II**	**Regency Ballroom** **Crystal Ballroom**

Session 6: Organoids for Studying Neuronal Disease		**Crystal Ballroom**
Moderators: Julio Martinez-Trujillo (Western University) and Ian Tobias (University of Guelph)		
2:15 – 2:40 PM	Liliana Attisano, Professor, University of Toronto *"Developing more complex models of cerebral organoids"*	
2:40 – 2:55 PM	Brandon Iturralde, University of Guelph *"Modeling Early Human Cerebellar Fate Specification in hiPSC-Derived Organoids"*	
2:55 – 3:20 PM	Marcelo Vazquez, Radiobiology Section Head, Canadian Nuclear Laboratories *"Brain Organoids Supporting Space Radiation Research"*	
3:20 – 3:45 PM	Yun Li, Scientist, Sick Kids *"Investigating mTOR hyperactivation related neurodevelopmental disorders in human brain cells and organoids"*	

3:45 – 4:30 PM	Award Presentations and Closing Remarks

End of Day 2

Keynote Speaker

Hans Clevers, MD, PhD
Professor of Molecular Genetics, Utrecht University

Hans Clevers is world-renowned for his work in the fields of cell biology, molecular signaling and stem cells. His research groups' discoveries include the detailed characterization of the molecular effectors and integrators of the "Wnt" pathway, which play crucial roles in health and disease, including in stem cells, regeneration and cancer. His group provided important insights into (intestinal) stem cell biology, exploiting LGR5 as a novel stem cell marker. This eventually led him to pioneer "organoids", 3-dimensional in vitro structures that behave anatomically and molecularly like the organ from which they are derived. Organoid biology has revolutionized the way we understand and approach human biology and medicine.

He is the recipient of multiple international scientific awards, including the Breakthrough Prize in Life Science. Hans Clevers is a member of the Royal Netherlands Academy of Arts and Sciences (NL), the National Academy of Sciences (USA), the Royal Society (UK) and the Academie des Sciences (France). He is also Chevalier de la Légion d'Honneur and Knight in the Order of the Netherlands Lion, among many other international accolades.

Invited Session Speakers

Liliana Attisano is a Professor in the Department of Biochemistry, with a laboratory based in the Donnelly Centre. Dr. Attisano's lab is focused on studying the molecular events that underlie signalling cascades such as TGFβ, Wnt and Hippo and elucidating how cells interpret contextual cues to control complex biological responses. Her lab uses cells, mice and most recently human stem cell-derived organoid models to better understand how disruptions in signalling pathways underlie human disease processes including cancer and neurological disorders. Dr. Attisano established the Applied Organoid Core Facility located in the Donnelly Centre, whose mission is to produce and provide organoids to users as well as holding workshops and providing one-on-one training in stem cell culturing and organoid generation.

François Boudreau is a Professor in the Department of Immunology and Cell Biology in the Faculty of Medicine and Health Sciences at the Université de Sherbrooke, Québec, Canada. His work focuses on defining the molecular mechanisms involved in gene transcription during normal intestinal development and diseases. He has identified transcription factors regulating intestinal gene expression and involved in the maintenance of intestinal epithelial functions, in colorectal cancer, inflammatory bowel diseases and metabolic disorders, notably diabetes and obesity. Prof. Boudreau's laboratory aims are to define the molecular cascades and the intestinal epithelium deregulated functions associated with the activity of these regulators, to identify novel targets for pharmacological and therapeutic applications. He is currently the Canadian Association of Gastroenterology (CAG) President.

Justin Chun is a nephrologist and Associate Professor of Medicine at the University of Calgary. He obtained his MSc and PhD in Cell Biology at the University of Alberta and a medical degree from the University of Calgary. He then completed an internal medicine residency and nephrology clinical fellowship at the University of Calgary followed by a post-doctoral research fellowship at the Beth Israel Deaconess Medical Center and Harvard Medical School. He is the Co-Director of the Human Organoid Innovation Hub and Assistant Director of the Precision Medicine Program in Nephrology in the Snyder Institute for Chronic Diseases. Dr. Chun's current research interests include diabetic kidney disease and bioengineering stem cell derived kidney organoids for regenerative medicine, disease modelling and drug testing.

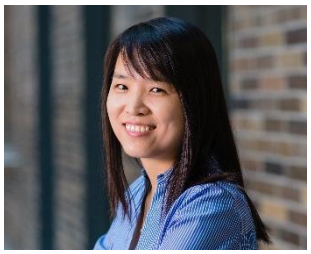
Hyunjae Chung received his PhD in Immunology from the University of Calgary and completed his postdoctoral training in Dr. Justin Chun's lab, where he studied kidney development and diabetic kidney disease using patient-derived kidney organoids. He is currently a senior stem cell scientist at HOIH, leading projects on iPSC reprogramming and iPSC-based disease modeling.

Jessica Esseltine is an Associate Professor and Heart and Stroke Foundation New Investigator at the Memorial University of Newfoundland and Labrador. As the iPSC lead for the NL sudden cardiac death research team, Dr. Esseltine combines patient-derived iPSCs with genetic engineering to examine inherited heart disease in Newfoundland families. Dr. Esseltine is currently funded by CIHR, Heart and Stroke, NSERC and the Stem Cell Network. Outside of work, Dr. Esseltine enjoys hiking the beautiful East Coast Trail, reading fantasy fiction, and playing with her 2 beautiful sons.

Véronique Giroux is an Associate Professor in the Department of Immunology and Cell Biology at the Université de Sherbrooke. Her research program focuses on understanding the molecular mechanisms underlying the functions of stem cells in the GI tract. The overarching goal of her laboratory is to develop new therapeutic strategies for digestive diseases by targeting specifically stem cells and their distinct features. She is also strongly committed to graduate student mentoring. She was awarded the RECMUS Prize in 2023 by the Faculty of Medicine and Health Sciences in recognition of the quality of her mentoring. She was recently appointed as the Director of the Cell Biology Graduate Studies.

Simon Hirota is a Professor and Associate Dean Research (Infrastructure) at the University of Calgary in the Snyder Institute for Chronic Diseases. His team's research interests are broad, spanning from understanding the role microbial metabolites in the regulation of intestinal mucosal homeostasis, elucidating the mechanism(s) driving intestinal fibrosis and pathogenic tissue remodelling in Crohn's disease, to characterizing the interplay between various mucosal immune cells and IL-22 in the context of health and disease. Dr. Hirota is the co-lead on the Canadian National Organoid Network and the director of the Snyder Institute's Human Organoid Innovation Hub.

Kalina Kamenova is the Founder and Research Director of the Canadian Institute for Genomics and Society. Dr. Kamenova is an engaged scholar specializing in research ethics, participatory methodologies, science policy, and public communication within the interdisciplinary domain of science and technology studies (STS). Her research critically addresses the ethical, social, and legal dimensions of biomedical innovation, with a particular focus on genomics, precision medicine, stem cell and regenerative therapies, and artificial intelligence in healthcare. Through an integrated approach that combines bioethics, public engagement, and policy analysis, Dr. Kamenova contributes to the development of evidence-informed frameworks for the responsible governance of emerging biotechnologies. She is the founder of Genomics4S, an independent research institute in Toronto, which fosters interdisciplinary collaboration among academic researchers, scientists, policymakers, and industry partners to advance ethical and socially responsive innovation.



Yun Li is a neurobiologist interested in understanding human brain development and diseases. She is currently an Assistant Professor at the University of Toronto, a Senior Scientist at the Hospital for Sick Children, and a Medicine by Design Investigator. Her laboratory studies how the human brain forms, what makes it unique from those of other species, and how disorders like autism impact its development and function. Her group takes the experimental approach of modeling human brain development in the dish, utilizing a combination of pluripotent stem cell technology, CRISPR/Cas9-mediated genome engineering, and 3D brain organoids.

Van Lu is an Assistant Professor in the Department of Physiology and Pharmacology at the University of Western Ontario. She completed her PhD in Pharmacology at the University of Alberta and pursued post-doctoral training at the NIH (USA) and the University of Cambridge (UK). Her work focuses on nutrient-sensing mechanisms in the gut hormone releasing cells of the intestinal epithelium, the enteroendocrine cells. Using human intestinal organoid models expressing genetically encoded biosensors and traditional electrophysiological techniques, she has elucidated novel signaling mechanisms that can be exploited to regulate endogenous hormone release. Recently, her lab has expanded their research into investigating secretory mechanisms in other gastrointestinal epithelial cells such as Tuft cells.

Shinichiro Ogawa is a Scientist at the McEwen Stem Cell Institute, University Health Network, and an Assistant Professor in the Department of Laboratory Medicine and Pathobiology at the University of Toronto. Dr. Ogawa's research team focuses on the differentiation of human pluripotent stem cells (hPSCs) into liver progenitor cells capable of generating functional hepatocytes and bile duct epithelial cells. His ongoing investigations utilize hPSC-derived liver cells and organoids/tissues to evaluate hepatic function restoration in experimental models, with the ultimate goal of developing cell- and tissue-based therapies as alternatives to liver transplantation for patients with liver failure. Additionally, Dr. Ogawa's research explores the use of hPSC-derived bile duct epithelial cells to model Cystic Fibrosis Liver Disease (CFLD) and conduct drug screening for potential treatments. Dr. Ogawa's work also extends to tissue engineering, where he is advancing the application of 3D bioprinting technologies to fabricate iPS cell-derived liver tissue, including the creation of 3D bile duct tubes from hPSCs. These innovative models serve as a foundation for drug screening and therapeutic approaches aimed at liver diseases.

Nikolina Radulovich, PhD, is a Staff Scientist at the Princess Margaret Cancer Centre in Toronto, where she leads the Living Biobank Organoid Team. She develops and biobanks patient-derived organoid models that help researchers study tumor biology, test drug responses, and explore resistance to therapies. Her work supports collaborative cancer research and has been featured in numerous publications on cancer biology and 3D cell culture technologies.

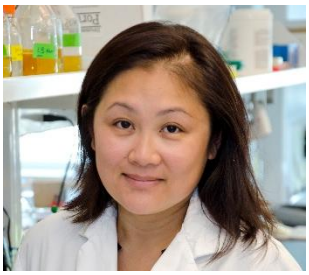
Jacob Shelley is an Associate Professor with a joint appointment in the Faculty of Law and the School of Health Studies in the Faculty of Health Sciences. He is co-director of Health Ethics, Law & Policy (HELP) Lab at the University of Western Ontario. His primary area of interest is the proper limits and role of law in promoting public health and preventing chronic disease. In addition, he is generally interested in issues that arise at the interface of law, health science, and ethics.

Marcelo Vazquez is the current Section Head, Radiobiology branch at the Canadian Nuclear Laboratories. He holds a MD and a PhD degree in Neurobiology and Radiobiology from the Universidad Nacional de la Plata, Argentina. His background is focused on neurosciences, radiobiology, radiation oncology, and space radiobiology. He has extensive experience utilizing particle accelerators to irradiate cells and animals to support space radiation research. He worked for Columbia University, NASA, Brookhaven National Laboratory, Baylor College of Medicine, and Loma Linda University. At CNL, he is leading several scientific projects and scientists in the study of low dose of space radiation supporting the Canadian Space Agency space program.

Bruce Vallance is a Professor in the Division of Gastroenterology, Department of Pediatrics at the University of British Columbia and CH.I.L.D. Foundation Chair in Pediatric Gastroenterology. His research addresses the mechanisms underlying Inflammatory Bowel Diseases (IBD) as well as the role played by enteric bacteria in controlling health and disease within the gastrointestinal tract. More recently, Dr. Vallance has focused on gut organoids, using them to model microbe-host interactions at the human intestinal mucosal surface. Based on this work, he was named the director of the Gut4Health Microbiome Core and co-lead of both the TRIANGLE national GI and Liver Training program and the CANadian Organoid Network (CNON). In 2023, he was awarded the Faculty of Medicine's Distinguished Achievement Award for Excellence in Basic Science Research.

Franco J. Vizeacoumar earned his PhD in Cell Biology from the University of Alberta and completed postdoctoral training in Systems Biology at the University of Toronto. He leads a research program dedicated to developing new therapies for solid tumors by exposing and exploiting the hidden weaknesses of cancer cells, using the genetic principles of synthetic lethality and synthetic dosage lethality. At the heart of his work is an ambitious vision: to create a comprehensive genetic map that reveals how cancer cells depend on specific gene interactions for survival. By charting these networks, his team aims to uncover the critical vulnerabilities that can be transformed into powerful therapeutic targets. The Vizeacoumar Lab's innovative research is supported by both national and provincial agencies, including CIHR, CFI, TFRI, CRS, SHRF, the Saskatchewan Cancer Agency, the College of Medicine at the University of Saskatchewan, and by philanthropic partners such as the Be Like Bruce organization.

Darcy Wagner joined McGill in January 2024 as the Canadian Excellence Research Chair in Lung Regenerative Medicine with a co-appointment in Medicine and Biomedical Engineering. The broad interest of her laboratory is the development of bioengineering strategies to generate lung tissue ex vivo as well as to develop new therapies to regenerate tissue in vivo. Her laboratory combines novel biomaterials with advanced manufacturing approaches to generate this tissue including the use of microfluidics and 3D bioprinting. Her laboratory was the first to 3D bioprint human airways containing regionally specified primary cells using tissue-specific bioinks. The long-term goal of the lab is to develop transplantable tissue to meet the unmet clinical need for solid organ transplantation but also to use these bioengineering advances to develop new ex vivo humanized models of disease, including organoids, to further our understanding of disease and to screen new therapies.

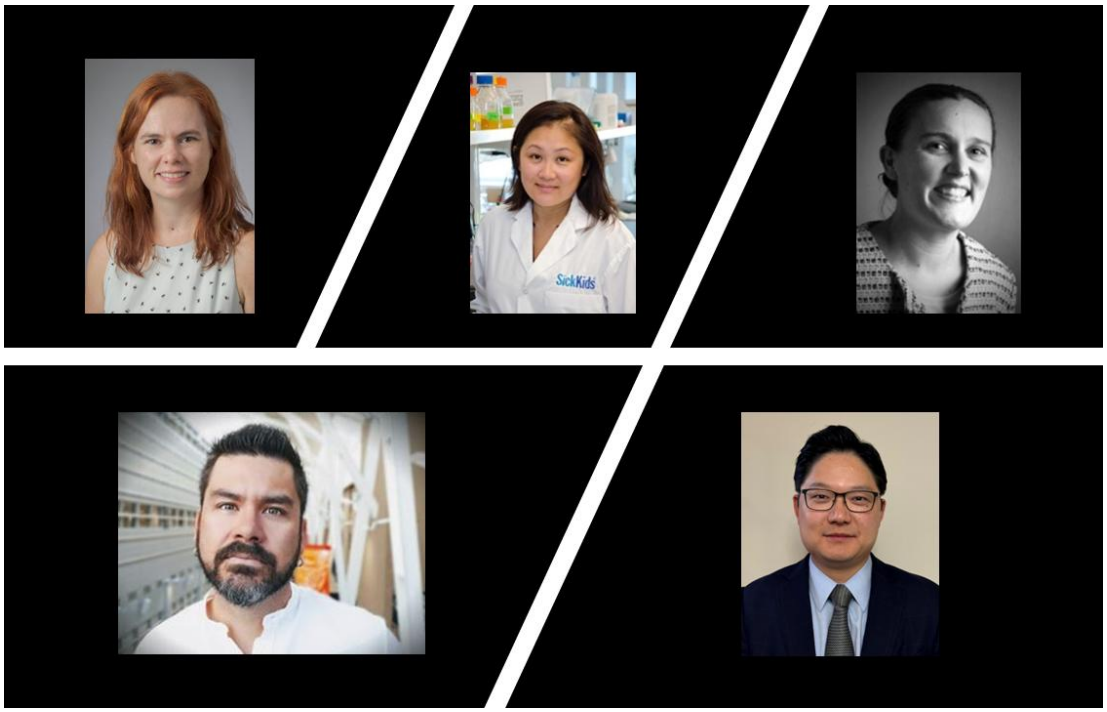


Amy Wong is a Scientist at the Hospital for Sick Children and an Assistant Professor at the University of Toronto. She pioneered the first method to generate mature airway epithelia from human stem cells, enabling disease modeling and drug testing for Cystic Fibrosis (CF). In recognition of her contributions to lung health research, she was awarded the King Charles III Coronation Medal by the Governor General's Office of Canada and the Masha Morton Award for excellence in CF research. Internationally recognized for her leadership in stem cell-based lung research, Dr. Wong's lab focuses on three key areas: 1) identifying genetic and molecular regulators of lung development and disease; 2) evaluating new genetic and cell-based strategies for CF precision medicine; and 3) developing physiologically relevant pre-clinical lung models to improve disease modeling and evaluate regenerative therapies.

Boyang Zhang is an Associate Professor in the Department of Chemical Engineering and an Associate Member of both the School of Biomedical Engineering and the Centre for Discovery in Cancer Research at McMaster University. He received his BSc in Chemical and Biomolecular Engineering from the Georgia Institute of Technology in 2010 and his PhD in Chemical Engineering and Applied Chemistry from the University of Toronto in 2016. His research focuses on developing automated, high-throughput 3D tissue culture platforms to support drug discovery and preclinical testing. He is also the Founder and CEO of OrganoBiotech, a startup company that commercializes tissue culture technologies developed in his lab for broader application in pharmaceutical research and development.

Meet the Speakers Networking Event

Would you like to meet with Drs. Justin Chun, Véronique Giroux, Simon Hirota, Darcy Wagner or Amy Wong? Sign up at the registration desk to meet them for breakfast on November 6 (7:30 – 8:30 AM). Spots limited so sign up early!



Session Speakers Selected from Submitted Abstracts

Fatemeh Ahmadi is a biomedical engineer and postdoctoral fellow at the University of Toronto. Her research focuses on developing automated microfluidic platforms for single-cell analysis and creating patient-derived tumor models to study drug response and resistance mechanisms. By combining bioengineering, imaging, and computational analysis, she aims to build data-driven tools that improve how we understand and treat cancer. Fatemeh's interdisciplinary work bridges engineering innovation and clinical research to make precision medicine more predictive, accessible, and impactful.

Eugenia Flores-Figueroa, PhD (UNAM), is a Scientific Associate at the Notta Lab, UHN, Toronto. She studies pancreatic cancer and leukemia using spatial technologies and DNA/RNA sequencing. Her work includes identifying a pro-inflammatory environment in pre-leukemia, mapping stem cell organization in aging bone marrow, and developing a 130-sample pancreatic cancer PDO dataset. With 30+ publications, she advances understanding of pancreatic cancer and hematopoiesis and collaborates with experts including Drs. John Dick, Stephanie Xie, Gregory Schwartz, and Federico Gaiti from UHN.

Brandon Iturralde is an MSc candidate in Biomedical Sciences within the Tobias Lab at the University of Guelph. He completed his undergraduate degree in Honours Life Sciences at McMaster University. His research interests include cell biology, genetics, and developmental biology. Brandon's current work focuses on early cerebellar development, using stem cell-derived models to study the molecular and cellular mechanisms that guide brain regionalization and progenitor diversification. His research is supported by the International Brain Research Organization (IBRO).

Darasimi Kola-Ilesanmi is an MSc student in Gastrointestinal Sciences at the University of Calgary, Cumming School of Medicine. Her research examines epithelial plasticity and spatial remodelling in human intestinal organoid models of Crohn's disease. She holds a BSc in Biochemistry and Molecular Biology from UBC Okanagan and has expertise in molecular cloning, live-cell imaging, and organoid-based disease modeling.

Ali Mousavi is a postdoctoral fellow in the Department of Surgery, Faculty of Medicine and Health Sciences at McGill University, and at the Research Institute of the McGill University Health Centre, under the supervision of Dr. Renzo Cecere. He currently holds a prestigious fellowship from the Quebec Research Fund – Health Section (FRQS). He completed his PhD in Biomedical Engineering at the University of Montreal and obtained his B.Sc. and MSc degrees in Chemical Engineering from Sharif University of Technology. His research primarily focuses on biomaterials, tissue engineering, 3D bioprinting, organ-on-a-chip technology, and stem cells

Kate Walton is a Research Assistant Professor at the University of Michigan and is the Associate Director of the Translational Tissue Modeling Lab working to make human lung and intestinal organoid models accessible to all researchers. Kate earned her PhD at Duke University in the lab of Dr. David McClay studying endomesodermal specification in the sea urchin gut and was a post-doctoral fellow in the lab of Dr. Deborah Gumucio studying cell signaling between the epithelium and mesenchyme in the developing mouse intestine. Her current work focuses on the development and maintenance of the villi in the human small intestine.

Instructions to Poster Presenters

- Please make your poster no larger than 4 ft x 4 ft (120 cm x 120 cm) in order to fit the display boards.
- Posters will be on display for the entire conference. Please display your poster by **noon on Nov 5**.
- Poster judging will take place during the following times:
    - Poster Session I (numbered posters): Nov 5, 1:30 – 2:30 PM
    - Poster Session II (lettered posters): Nov 6, 1:15 – 2:15 PM
- Please be beside your poster during this time.
- Remove your posters at the end of Poster Session II on Nov 6.

Instructions to Poster Judges

- Please sign-in with a team member at the Registration desk to receive your judging assignments and QR code.
- We are using an electronic scoring submission system, so please ensure you have an electronic device (mobile phone, tablet, laptop, etc.) to enter scores.
- A team member at the Registration desk will be available to assist input of scores, if needed.

Audience Choice: Best Poster Prize!

We are also awarding a prize to the best poster presentation voted by the audience! A voting ballot will be sent via email during the conference.

Voting closes **Nov 6 at 2:30 PM**.

Have your say to vote for the best poster at CANCOR!

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Abstracts Poster Session I

1. **Optimizing porosity and mechanical strength of photo-cross linkable biomaterials for growth of intestinal organoid cultures**

Abbey J. Kirkwood[1,5], Alap A. Zahid[2], Aishik Chakraborty[2], Frederikke Larsen[3], Samuel Asfaha[4,5], Arghya Paul[2] and Van B. Lu[1,5]

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Abstract:
Organoids have emerged as powerful tools in biomedical research, serving as physiologically relevant 3D models to study tissue development and disease. Their successful generation and growth rely on supportive 3D matrices that mimic the extracellular matrix (ECM), in which they are embedded. Currently, the most widely used scaffold is Matrigel®, a complex and undefined mixture of ECM proteins. However, its batch-to-batch variability often compromises structural integrity and impairs the diffusion of growth factors, leading to inconsistent organoid viability and differentiation.

This project seeks to overcome these limitations by developing novel hydrogels based on gelatin methacryloyl (GelMA). GelMA is a photo-crosslinkable biomaterial known for its biocompatibility, adjustable mechanical properties, and reproducibility. GelMA can be engineered to mimic tissue-specific ECM and is compatible with 3D bioprinting technologies, making it a promising alternative for organoid culture.

We aim to design GelMA hydrogels with improved permeability and mechanical strength to support robust organoid growth and function. Specifically, we will adjust GelMA composition and crosslinking parameters to increase pore size and change the geometries in which hydrogels are bio-printed to minimize diffusion distances. Together, these modifications aim to enhance the delivery of nutrients and signaling molecules to the matrix core, promoting healthier and more consistent cultures.

Hydrogel properties will be thoroughly characterized: pore size by electron microscopy, permeability by dye diffusion assays, and tensile strength by compression testing. Functional validation will be performed using intestinal organoids. Ultimately, this project will provide improved scaffolds that enable healthier, more reliable organoid cultures. By addressing the limitations of current ECM substitutes, this work has the potential to significantly advance organoid-based research and accelerate its applications in personalized medicine.

2. Development of a 3D beating heart-on-a-chip for modeling dilated cardiomyopathy using patient-specific stem cells

Ali Mousavi (1,2,3,4), Gregor Andelfinger (2), Renzo Cecere (4), Houman Savoji (1,2,3)

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2 CHU Sainte-Justine Research Center, Montreal, QC, Canada
3 Montreal TransMedTech Institute, Montreal, QC, Canada
4 Division of Cardiology and Cardiac Surgery, McGill University Health Centre, Montreal, QC, Canada

Abstract:
Dilated cardiomyopathy (DCM) is the most common reason for heart transplantation with a 50% risk of progression to heart failure within 5 years of diagnosis. Conventional disease modeling approaches (e.g., animal models and monolayer cell culture) fail to fully recapitulate the sophisticated structure and dynamic function of the human heart. Therefore, there is an urgent need for a physiologically relevant alternative model in the preclinical stage. Heart-on-a-chip (HOC) platforms enable real-time monitoring of disease progression, pathological mechanisms, and drug screening by creating miniaturized biomimetic engineered heart tissues (EHTs). Here, we developed a functional HOC model for personalized medicine using patient-specific human induced pluripotent stem cells (hiPSCs), reprogrammed from the patients' blood samples. The chip contains two cell seeding chambers with an array of flexible silicone pillar pairs to support tissue formation. Each device could be integrated into one well of 12-well plates for high throughput performance. The healthy and DCM hiPSCs were differentiated into cardiomyocytes (CMs), purified via lactate starvation, and characterized with flow cytometry and immunocytochemistry. An optimized ratio of ventricular human cardiac fibroblasts was further added to patient-derived hiPSC-CMs, and the cells were encapsulated in a fibrin/Geltrex hydrogel (containing fluorescent beads) and seeded in each chamber of the device. The tissue gradually compacted and started beating spontaneously. Immunofluorescent staining of cardiac-specific biomarkers revealed structural abnormalities in DCM tissues, such as reduced cell alignment and elongation. The tissue functional responses (e.g., calcium transient and beating) were investigated based on pillar deflection and bead movement after two weeks of culture, showing ventricular tachycardia in DCM tissue and highlighting the functional hallmarks of the disease. The tissue contractility was also mapped in real-time based on the bead movements using a custom-built MATLAB code, showing the heterogeneity in contractile stress in different parts of an engineered heart tissue. Bulk RNA sequencing was performed on these 3D microtissues, which demonstrated gene expression changes in the DCM group and identified specific biomarkers associated with the disease. The platform was further validated using two drug candidates (norepinephrine and lidocaine), and their expected chronotropic and inotropic effects were successfully observed. Notably, norepinephrine treatment partially restored the early afterdepolarization-induced arrhythmic patterns in DCM tissue. This HOC model successfully mimics key aspects of DCM at structural, functional, and molecular levels. Its responsiveness to drug treatment underscores its potential for disease modeling, drug screening, and personalized medicine. The platform offers a powerful tool to explore cardiac pathophysiology and support the development of targeted therapies to serve as a preclinical tool for evaluating therapeutic efficacy and safety in a patient-specific context.

3. Incorporating immune cells in a patient-derived liver organoid model to understand the immune drivers of liver disease

Amelia Montemarano[1], Jawairia Atif[2], Sharon Hyduk[3], Ariya Shiwram[2], Michelle Sue[3], Feng Xu[4], Diana Nakib[2], Justin Manuel[3], Xue-Zong Ma[3], Ian McGilvray[3], Sonya MacParland[1,2,3]

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2 Dept. of Immunology, University of Toronto
3 Ajmera Transplant Centre, University of Toronto
4 University of Toronto

Abstract:

Introduction: Chronic liver diseases pose a major global health burden, accounting for nearly 2 million deaths annually. These conditions are difficult to treat because their complex, long-term pathogenesis cannot be fully captured in current models. Conventional 2D hepatocyte cultures fail to replicate the liver's 3D architecture, cell–cell interactions, and functional capacity, resulting in poor predictive power for human disease and drug responses. Consequently, effective therapies remain limited. Recently, patient-derived liver organoids (PDOs) have emerged as promising 3D models that better recapitulate the structure and function of human liver tissue. Yet, current organoid systems lack resident immune cells, constraining their use in studying immune-driven mechanisms of liver disease.

Objective: PDOs retain many patient-specific epithelial features but lack the liver's immune microenvironment. To bridge this gap, we aim to adapt PDO systems to incorporate autologous macrophages through two methods: 1) retaining macrophages plated at organoid seeding from total liver homogenate (TLH) through the optimization of growth conditions, and 2) supplementing autologous macrophages at passage of established organoids. Within these macrophage-supplemented organoids, we plan to investigate how different macrophage phenotypes, such as inflammatory, reparative, and lipid-associated macrophage (LAM)-like cells, impact organoid phenotype and function. This platform will enable mechanistic studies of macrophage-driven pathways in liver health and disease and improve PDO utility for translational research.

Methods: We generated hepatic organoids from TLH of perfused caudate lobes from healthy donors. Organoids were seeded in Matrigel and expanded using a medium developed by Huch et al. to stimulate liver progenitor proliferation. Live, whole-mount imaging was used to visualize the incorporation of macrophages (CD45, CD68) into EpCAM+ organoid cultures. We tested several concentrations of macrophage colony stimulating factor (M-CSF) in the PDO culture medium to examine its impact on primary macrophage survival in organoid culture. For the macrophage-supplemented organoids, $CD14^+$ monocytes isolated from peripheral blood or liver tissue will be differentiated in vitro. An Inflammatory-like phenotype will be induced with IFN-γ and IL-1β, an alternatively-activated polarization with TGF-β and IL-10, and LAM-like macrophages with acetyl-LDL. These defined populations will then be introduced into dissociated PDOs during passage, embedded in Matrigel, and tracked in culture. Macrophage and organoid phenotype will be examined over time using flow cytometry and immunofluorescence (IF), alongside effects on organoid growth, viability, transcriptional profile, cytokine secretion, and functional readouts (cell polarity, forskolin-induced swelling).

Results: Using IF, we did not observe an appreciable difference in the number of liver macrophages associating with PDOs with the addition of M-CSF in culture medium. By adding liver macrophages at passage, we were able to observe supplemented macrophages integrating with PDOs using IF. Building on these findings, we will incorporate differentially polarized macrophages into PDOs to investigate how distinct macrophage states influence organoid phenotype and function.

Impact: By incorporating macrophage with defined phenotypes into PDOs, we aim to capture the diverse roles of macrophages in shaping liver health and disease. This macrophage-enriched PDO platform will enable mechanistic studies of how inflammatory, reparative, and LAM-like macrophage states influence liver epithelial, stromal, and immune function. Ultimately, this model will provide a patient-specific tool for investigating immune-driven liver diseases and testing macrophage-targeted therapeutic strategies, with the long-term goal of improving clinical outcomes and reducing the need for liver transplantation.

4. **IL4-treated macrophages enhance epithelial barrier function and muc2 gene expression in human intestinal organoids**

Blanca E. Callejas, James A. Sousa, Arthur Wang, Ryan Rosentreter, Simon Hirota, Remo Paniconne and Derek M. McKay

Abstract:
Inflammatory bowel diseases (IBD) are characterized by compromised epithelial barrier that can allow harmful substances in the lumen to infiltrate the lamina propria, driving inflammation. Individuals with IBD experience a reduced quality of life, and while treatments have improved, a cure focused on preserving the epithelial barrier remains elusive. Interleukin-4 (IL-4)-differentiated macrophages (M(IL4)) from healthy donors and individuals with Crohn's disease (CD) have been shown to reduce the severity of murine colitis and promote wound repair in monolayers of human colon-derived epithelial cell lines, supporting the potential of autologous M(IL4) therapy. While encouraging, it remains unclear whether M(IL4)s would protect the barrier function of primary gut epithelia.

We investigated the ability of M(IL4) to preserve epithelial barrier function in 3D human inflamed organoids. Monocyte-derived M(IL4)s from healthy volunteers exhibited increased CD206, RAMP1 and CCL18, along with decreased expression of CD14 mRNA. Exposure to a cocktail of pro-inflammatory cytokines, IFN-γ, TNFα, and IL-1β (all at 10 ng/ml for 24h) disrupts epithelial barrier of 3D human organoids as defined by influx of a 4KDa FITC-dextran. Conditioned media (CM) from M(IL4) added to cytokine-treated 'inflamed' colonoids significantly reduced the epithelial barrier defect. Notably, M(IL4)-CM also promoted the expression of goblet cell-associated mucin, MUC2 at both protein and mRNA levels. At the same time mRNA expression of the canonical stem cell factor, LgR5, was reduced by M(IL4)-CM.

Overall, these preliminary data suggest that human M(IL4)s enhance epithelial barrier function and support the autologous transfer of M(IL4)s as a therapeutic strategy for IBD.

5. Modeling Early Human Cerebellar Fate Specification in hiPSC-Derived Organoids

Brandon Iturralde, Department of Biomedical Science, University of Guelph

Abstract:
Background: Long considered as a balance and motor control center, the human cerebellum contains the majority of the brain's neurons and plays important roles in cognition, emotional processing, and behaviour. Guided human induced pluripotent stem cell (hiPSC) differentiation protocols have enabled the generation of cerebellar organoids that recapitulate many important steps that establish a blueprint for future structural elaboration and functional diversification of the developing cerebellum. These include the generation of signaling organizers that pattern the hindbrain territory, namely the mid-hindbrain boundary, and the formation of germinative zones such as the rhombic lip that specify neural progenitor cells (NPCs). However, the fundamental sequence of gene regulatory events and the factors regulating cell type diversity in the human cerebellum remain elusive. Even subtle disruptions to the normal developmental programming of the early cerebellum can result in structural malformations, errors in NPC specification or differentiation, or affect neuron survival and connectivity, leading to the manifestation of neurodevelopmental disorders. By integrating bulk gene expression profiling to mark distinct stages of cerebellar patterning in a hiPSC-derived organoid model and single-cell RNA sequencing (scRNA-seq) to examine the heterogeneity of cerebellar identities, it is possible to illuminate the timing and gene regulatory logic underpinning early human cerebellar cell differentiation.

Hypothesis & Objectives: I hypothesize that exposing hiPSC-derived neural organoids to cerebellum patterning factors at defined intervals will induce temporally and spatially regulated transcriptional programs for all major cerebellar lineages and early born cell types.

Objective 1: Quantify expression of key cerebellar transcription factors (EN1, EN2, GBX2, SOX2) using reverse transcription quantitative PCR (RT-qPCR) and immunofluorescence to define early signatures of fate commitment.
Objective 2: Characterize the diversity of progenitor cell states and identify transcriptional programs driving cerebellar fate specification using single-cell RNA sequencing.

Methods: Human cerebellar organoids were generated under defined conditions designed to suppress extraneous germ layer fates and promote the onset of mid-hindbrain identity. Neural induction was guided by dual-SMAD inhibition, followed by activation of WNT signaling and exposure to FGF8b. Organoids were harvested for total RNA at multiple stages (days 4, 8, 12, 16, and 28) capturing the transition from neural induction through mid–hindbrain patterning to early cerebellar fate specification for bulk RT-qPCR (n =3-6). To visualize the spatial organization of neural progenitor cells, immunofluorescence microscopy was carried out using antibodies targeting cerebellum development markers (EN1, GBX2, SOX2, PAX6). To capture the onset of gene regulatory network activation in cerebellar NPCs, scRNA-seq was performed for days 15 and 19 organoids (n=2 per timepoint) using particle-templated instant partitions (PIPs), a droplet emulsion technique that enables accurate capturing and barcoding of individual cells.

Results & Impact: From the scRNA-seq data, I identified 11 distinct cell populations including mid-hindbrain boundary, choroid plexus, and two compartments of the rhombic lip. I observed the initial emergence of rhombic lip NPCs in day 19 organoids, marked by the co-expression of PAX6 and ATOH1, following a conserved transcriptional logic seen in animal models. Pseudo-bulk analysis of the distinct upper and inner rhombic lip cells suggests that Notch signaling interactions (DLL1, MFNG) are temporally linked to the activation of essential neuron differentiation machinery (SRRM4, STMN2). These findings advance our understanding of NPC specification and neuron differentiation in the early human cerebellum, which may shed light on molecular mechanisms affected in developmental disorders.

6. Bioengineered 3D hPSC-cholangiocyte ducts with physiological signals for biliary disease modelling

Britney Tian[1,2], Mina Ogawa[1], Manami Kondo[1,2], Gabriella Langeveld[3], Ling Jun Huan[3], Feng Zhang[4], Andrew Hollinger[4], Sara Deir[4], Christine Bear[3,6], Boyang Zhang[4,5], Shinichiro Ogawa*[1,2,7,8].

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Abstract:
Background: Despite recent advances in cholangiocyte organoid systems, no human cell-based platform yet faithfully recapitulates intrahepatic cholangiocyte physiology in vitro, limiting our ability to model complex biliary disease pathogenesis. Human primary or induced pluripotent stem cell (h/iPSC)-derived cholangiocyte organoids have been used for disease modelling, such as cystic dysfunction in primary sclerosing cholangitis patient iPSC-derived extrahepatic organoids, and functional defects resulting from CFTR mutations in cystic fibrosis patient iPSC-derived intrahepatic organoids. Although these models can capture key aspects of biliary physiology and disease, they lack the open lumen of intrahepatic networks as seen in vivo, restricting investigation into the complex interplay between biliary constituents and fluid flow. Intrahepatic cholangiocytes are continuously exposed to a concentrated stream of bile acids (BAs), and a recent study by Sampaziotis et al. (2020) sheds light on the critical role of BAs as niche signals to shape cholangiocyte spatial identity. In disease contexts, the composition of the BA pool can become dysregulated; yet the effective intrahepatic BA concentrations and the specific physiological and pathological contributions of individual BA species remain poorly defined, highlighting the need for physiologically relevant in vitro platforms that can systematically interrogate their effects. Advances in bile-duct-on-chip platforms overcame the limitations in conventional 3D organoids by offering a perfusable lumen to support fluid flow and the controlled introduction of physiological biliary constituents and supporting cell types that act as key regulators to support biliary epithelium function. However, these studies mostly utilized murine or primary patient cell lines, and no study has yet systematically integrated these factors into a human intrahepatic cholangiocyte–based microfluidic platform to examine cholangiocyte response.

Results: To enable the combinatorial testing of multiple factors that more accurately replicate the complex biliary environment under physiologically relevant fluid flow, we engineered a perfusable, hPSC-derived 3D bile duct in within the AngioPlate384 platform. This device can hold up to 128 tissues within a single plate to enable the parallel testing of multiple complex biliary microenvironmental signals and features an open-top design to allow easy tissue retrieval for downstream flow cytometry and molecular profiling. Within the tubular system, we efficiently differentiated hepatoblasts (hPSC-HBs) of above 98% purity into over 95% cytokeratin-7 positive cholangiocytes by the end of maturation, displaying robust primary ciliation (>70% cilia-positive cells), apical-basal polarity and high cystic fibrosis transmembrane conductance regulator (CFTR) function, demonstrated by over 30% stimulated apical chloride conductance activity. We showed that fluid flow and stromal cell incorporation jointly enhances hPSC-cholangiocyte function, and that bile acid exposure, alone or in combination with a pro-inflammatory cytokine interferon-gamma, can synergistically modulate cholangiocyte responses by disrupt barrier integrity and primary ciliation and gene expression. Lastly, we incorporated proliferative murine or hPSC-derived hepatic mesenchymal cells to model biliary fibrosis, a key feature of biliary disease. Overall, our functional, ciliated hPSC-derived 3D cholangiocyte tubes represent a powerful platform to dissect how physiologically relevant signals preserve biliary epithelial homeostasis and how their disruption drives pathological changes. By integrating models of both healthy and disease-associated conditions, this system mechanistically evaluates cholangiocyte function to inform the development of individualized strategies and provides a foundation for advancing precision medicine approaches in biliary disease.

7. Simulating The Physicochemical Influences Of Amniotic Fluid In Human Intestinal Organoid Culture

Chaitali Chitnis (University of Michigan), Madeline Eiken (University of Michigan, Co-Founder of Intero Biosystems), Jason Spence (University of Michigan, Co-Founder of Intero Biosystems)

Abstract:
Human intestinal organoids (HIOs) are human iPSC-derived models of the gastrointestinal system, specific to the small intestine. HIOs are currently matured in Matrigel over the course of 28 days. However, this environment is far from representative of the gut's development in vivo both chemically and mechanically. Matrigel's composition is poorly defined, confounding the signaling environment of HIOs and limiting their translational reach. And, as the gut rotates out of the body into the surrounding amniotic fluid for several weeks during early development, its mechanical environment is not well simulated via a fixed matrix. Our work investigates alterations to the standard HIO culture protocol - forced aggregation techniques and motion-based, matrix-free suspension culture - and their impact on HIO formation, growth, and development.

After our 3-day definitive endoderm (Activin A + FBS) and 6-day hindgut (FGF4 + CHIR) differentiation protocols, spheroids that spontaneously lifted off the monolayers were plated in Matrigel and the residual monolayers were isolated and exposed to various forced aggregation techniques. The developing organoids were then patterned and assigned to one of several movement conditions in suspension culture for 28 days, after which they were analyzed via PCR and immunofluorescence staining. HIO morphology was also quantified over the 28-day period using a Python-based image analysis software.

Matrix-free suspension culture in microwells, combined with various forced aggregation techniques and styles of motion, creates organoids that maintain intestinal identity and organization with occasional variations in epithelial, mesenchymal, and neural cell population subsets. Morphologically, microwells allow for dramatically more consistent size evolution over time against the Matrigel standard, with remarkably similar day 28 growth plateaus across conditions. Preliminary data also demonstrated that motion-based suspension culture within microwells leads to atypical organoid shapes, likely due to boundary constraints.

Our work, thus far, illustrates the beginnings of a more developmentally accurate mechanical and chemical environment for HIO culture in vitro. Future directions include investigating additional variations to motion-based, matrix-free suspension culture, and assessing the impact of suspension culture viscosity and various amniotic fluid signaling factors on HIO maturity and development.

8. Developing patient-derived organoids from endoscopic ultrasound

Courtney Brooks, Emilie Jaune-Pons, Jennifer Campbell, Nadeem Hussain, Michael Sey, Brian Yan, and Christopher Pin

Departments of Physiology and Pharmacology, Oncology, Paediatrics and Medicine, Western University
Baker Centre for Pancreatic Cancer

Abstract:
Patient-derived organoids (PDOs) have been suggested as potential AVATARs for patient response to therapies, especially regarding cancer treatment. However, developing this system for pancreatic ductal adenocarcinoma (PDAC) is hampered by a number of factors. PDAC shows significant heterogeneity between and within patients, with several molecular subtypes described. There is also an extensive tumour microenvironment (TME) and dense stroma that includes cancer-associated fibroblasts (CAFs), which also showed heterogeneity between patients. Finally, PDAC shows rapid progression of the disease that requires immediate treatment after diagnosis. To circumvent these issues, we are developing a living biobank from fine needle biopsies obtained during endoscopic ultrasound (EUS). EUS procedures typically occur prior to treatment and are used to provide definitive diagnosis of PDAC, thereby generating tissue samples at the earliest time point in a patient's treatment journey. The goal of this study was to determine the utility for using EUS-derived PDOs for identifying patient-specific therapies for PDAC. A secondary outcome was to define culture conditions and technical approaches that will increase the success of organoid development.

Patients with a suspected PDAC were consented through the Baker Centre for Pancreatic Cancer Research as part of DERIVE (Determination of Response to Therapy in Individual Patients). One or two needle biopsies were obtained using a SharkCore 22G needle following an initial biopsy for pathological analysis. Biopsies were placed into Extreme DMEM/F12 + Glutamax + P/S + 0.1% BSA on ice, then minced and digested using the Miltenyi Human Tumour Dissociation Kit. The resulting cell slurry was grown basement membrane extracts (BME) with a portion plated directly on plastic to establish patient-specific CAF samples. PDO and CAF cultures were characterized by RNA-seq, flow cytometry and immunofluorescence for markers of basal and classical PDAC subtypes.

160 EUS biopsies were obtained for this study, 134 with a confirmed diagnosis of PDAC. 20 samples were lost due to contamination, while 13 samples showed no visible cores (i.e. all blood). From the remaining 101 cores, we obtained PDOs at a 44.6% success rate. At P3, we were able to perform whole exome sequencing and targeted mutational analysis. 10.9% of samples resulted in PDOs at P6. Mutational analysis showed a variety of KRAS and TP53 mutations. Histological analysis showed PDOs either appear as dense proliferative structures, globular-like cysts or a mixture of both. Purity Independent Subtyping of Tumours (PurIST) based on RNA-seq indicated PDOs were classical in nature but showed marked differences in gene expression. Flow cytometry and IF analysis showed a range of CAF subtypes between patients and identified cultures containing tumour cells based on KRAS mutational analysis and staining for EpCAM. In some cases, we obtained cancer cells from both 3D and 2D cultures. Finally, PDOs showed some differences in gemcitabine sensitivity, but this did not align to basal vs classical subtypes.

In conclusion, we have identified two approaches for obtaining PDOs from EUS sampling. When successful, PDOs can be expanded and characterized in a time frame that allows the identification of second line treatments for PDAC.

9. Partial EMT drives collective epithelial cell migration during Crohn's disease fistula formation: A human intestinal organoid model

Darasimi Kola-Ilesanmi, Matthew Stephens, Colin MacKenzie, Braedon McDonald, Frank Visser, Simon Hirota

University of Calgary

Abstract:
Background: Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract. One of the common complications of CD is the formation of fistulas, which are abnormal passages between the intestine to other organs or the exterior of the body. Fistulas occur in 30-50% of CD) patients and represent a major therapeutic challenge. Fistula tracts are lined with transitional cells exhibiting features of partial epithelial-to-mesenchymal transition (EMT), with retained epithelial cell markers and acquired invasive properties. The inflammatory microenvironment in CD is characterized in part by elevated TGFβ, TNFα, IL13 and IL22, which are the likely drivers of EMT. However, mechanisms remain unclear due to a lack of physiologically relevant models.

Aim: To establish a human intestinal organoid (HIO) model of CD cytokine-induced partial EMT and characterize the morphological and functional changes driving fistula formation.

Methods: Organoids were derived from ileal biopsies of healthy donors (n=4) and CD patients (n=2). Organoids were cultured in reduced Matrigel with 8-day combination cytokine treatment: TGFβ (20 ng/ml), TNFα (20 ng/ml), IL-13 (100 ng/ml) & IL-22 (100 ng/ml). Organoid morphology was monitored via live cell imaging (Incucyte SX5), gene expression quantified by RT-qPCR at day 8, and cytoskeletal organization assessed by F-actin staining.

Results: Cytokine treatment induced a partial EMT state in HIOs, characterized by concurrent epithelial identity and acquired mesenchymal features. By D4, organoids transitioned from 3D spheroids to flattened structures with sheet-like migration. PCA analysis of morphology metrics revealed progressive divergence from untreated controls, with Feret diameter and aspect ratio driving PC1 (71% variance). F-actin staining revealed filopodia, lamellipodia, and stress fiber formation indicative of a migratory state, with cell-cell contacts were retained during migration. RT-qPCR at D8 revealed E-cadherin (CDH1) and stem cell marker LGR5 were unchanged, while EMT markers SNAI2 showed strong upregulation (11-fold, $p<0.05$) along with ITGB6 (6-fold), ZEB1 (2.5-fold) and ETS1 (2-fold). Notably, SNAI1 was supressed (10-fold), as was αSMA (ACTA2; 2-fold), representing a response distinct from canonical EMT. Both healthy and CD organoids displayed similar changes, demonstrating the CD inflammatory environment alone is sufficient to induce EMT responses.

Conclusions: CD fistula-associated cytokines can induce a partial EMT state in HIOs. This hybrid state is characterized by SNAI2-dominant reprogramming and collective epithelial migration, with retained epithelial features and acquired migratory behaviour. This model provides a physiologically relevant platform for investigating EMT dynamics and identifying molecular targets to prevent fistula formation in CD.

10. Investigating the role of Snail independent of TGFβ signaling in epithelial ovarian cancer

Emily Tomas[1,2], Jennifer Davis[1], Yudith Ramos Valdes[1], Trevor G Shepherd[1,2,3,4]

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2 Anatomy & Cell Biology, Western University, London, ON, Canada
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Abstract:
Introduction: Epithelial ovarian cancer (EOC) metastasizes uniquely by spreading as spheroids within the peritoneal cavity. Our extensive research on EOC spheroid pathobiology has revealed dynamic changes in cell signaling that enable stress adaptation and survival, although the mechanisms remain unclear. Present work characterizing EOC spheroids versus organoids demonstrated differential signaling pathways confirming our speculations that cells undergo biological switching behaviours during metastasis. Additionally, prior studies identified elevated transforming growth factor beta (TGFβ) signaling in spheroids, facilitating epithelial-to-mesenchymal transition (EMT) for cell survival. However, recent transcriptomic analysis of seven patient ascites-derived (iOvCa) cell lines confirmed increased TGFβ signaling in spheroids compared to organoids; however, EMT was elevated in organoids compared to spheroids, presenting a paradox.

Methods: In this study, iOvCa cell lines were used to generate spheroids in suspension on Ultra-Low Attachment plates and organoids using modified patient-derived organoid culture conditions. RT-qPCR and immunoblotting of key markers for the TGFβ signaling pathway and EMT were conducted on spheroids in comparison to organoids. Manipulation of TGFβ signaling was performed by using the SB-431542 inhibitor and TGFβ1 ligand. siRNA knockdown of SNAI1 was completed on adherent and spheroid cultures to demonstrate the influence of Snail on EMT markers, spheroid reattachment and cell viability.

Results: In terms of EMT, there were low levels of Vimentin and inconsistent levels of E-cadherin and N-cadherin were observed in iOvCa spheroids in comparison to organoids. Consistently in all cell lines, there were high levels of Snail in spheroids and high levels of Claudin-1 in organoids. TGFβ inhibition and activation further demonstrated TGFβ's selective regulation of EMT proteins, indicating our iOvCa cells exhibiting a hybrid EMT phenotype regardless of biological state. A time course analysis of TGFβ signaling demonstrated early regulation of Snail in spheroids, except it is not sustained after 24 hours. Loss of SNAI1 in two iOvCa cell lines did not influence any of the EMT protein markers, but did decrease spheroid reattachment capacity and cell viability.

Conclusions: This contradictory TGFβ signaling and Snail activity within our spheroids and organoids may provide new insight into EMT and how it assists in EOC metastasis. Our results indicated that culture condition may influence Snail and Claudin-1 protein expression more than TGFβ signaling. Of course, additional experiments need to be completed to determine the exact mechanism of Snail regulation in EOC spheroids compared to organoids. Therefore, our findings emphasize the significance of using three-dimensional models to study disease progression and uncover novel therapeutic targets, especially for EOC.

11. Interpretable variational autoencoder for integrating epigenetic and transcriptomic profiles in Pancreatic Cancer

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Abstract:
Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers in the world, where survival rates remain dismal. Pancreatic cancer is driven by extensive inter- and intra-patient heterogeneity, along with marked resistance to existing therapies. A key gap in our understanding of PDAC is the role of epigenetic regulation in shaping tumor behavior and tumor microenvironment (TME). Among the key contributors to this regulation are cancer-associated fibroblasts (CAFs), particularly the inflammatory (iCAF) and myofibroblastic (myCAF) subtypes, which can influence tumor progression. Despite their recognized importance in modulating tumor growth and therapeutic response, the epigenetic impact of CAFs on tumor cells remains poorly understood.

To address these gaps, our laboratory has generated multi-modal data from patient-derived organoids (PDOs) treated with conditioned media from iCAF or myCAF, or human feeding media (HFM). HFM-treated PDOs, which lack cancer-associated fibroblasts, serve as a baseline to identify epigenetically modified regions influenced by TME. We have profiled PDOs +/- CAF-conditioned media using RNA-seq, ATAC-seq, DNA methylation, and whole-exome sequencing (WES). In parallel, access to clinical metadata allows us to directly align molecular characteristics with patient outcomes.

Leveraging this comprehensive dataset, we sought to uncover molecular signatures and regulatory mechanisms underlying PDAC heterogeneity. We developed a two-channel attention-based variational autoencoder (VAE) to identify and prioritize regulatory regions from ATAC-seq data binned around transcription start sites (500 kb). To account for spatial dependencies between genomic bins, we employed 1D convolutional layers to summarize chromatin accessibility along the genome. Recognizing CAF heterogeneity, iCAF and myCAF ATAC-seq profiles were modeled as distinct channels within the VAE, enabling integration into a shared latent space that reflects the complexity of the TME in organoids. Additionally, we incorporated an attention mechanism together with a regression head into our model to provide interpretable weights that highlight chromatin regions most predictive of transcriptional output for each CAF subtype.

Our model achieved a prediction accuracy of approximately 0.70 (P-value < 0.001) on this dataset. By interrogating attention weights which correspond to chromatin accessibility in the defined window, we are able to identify regulatory regions of each gene. For instance, we examined attention weights and ATAC read counts for KRAS, a key regulator in PDAC, and their product as a proxy for determining the candidate regulatory regions for each CAF subtype.

Additionally, we were able to pinpoint CAF-mediating transcription factors that regulate gene groups our model flagged as prime, epigenetically active yet transcriptomatically silent. Analysis of TF expression further indicates their presence in the PDOs examined in this study.

In summary, this interpretable deep learning framework advances our understanding of PDAC biology, particularly its epigenetic dimension, and offers a foundation for precision oncology by linking molecular subtypes to therapeutic response.

12. Microfluidic Platform for Organoids-on-Chip Culture and Experiments

Gavin Goebel*, Sehan Punchihewa*, Christopher Pin, Eugene Wong, Marcelo Vasquez, and Tamie Poepping (*co-first authors)

Abstract:
Organoids-on-chip technology represents a transformative convergence of tissue engineering and microfluidics, offering physiologically relevant in vitro models for studying organ function, disease mechanisms, and therapeutic responses. This project presents the design and fabrication of a novel microfluidic organoids-on-chip platform that integrates dynamic perfusion, real-time sensing, and tunable microenvironmental control to support long-term culture and monitoring of organoids. The chip is constructed using a biocompatible polydimethylsiloxane (PDMS) chamber bonded to glass, enabling scalable and reproducible device fabrication. A microfluidic channel flows beneath a central organoid chamber, providing perfusion to mimic vascular exchange and nutrient diffusion in native tissues. Organoids grown in Matrigel domes are cultured on a porous membrane within a small well of a thin acrylic insert that is then placed into the central chamber flush with the microfluidic channel. The insert separates the overlying cell media in the chamber from the perfusion media in the microchannel below. Utilizing a peristaltic pump, controlled flow through the device provides physiologically relevant shear stress, improving nutrient delivery, and waste removal while preserving organoid morphology. Compared to conventional static culture techniques, the microfluidic approach is expected to better sustain organoid viability and functionality by enabling continuous nutrient exchange, waste clearance, and microenvironmental control, which are difficult to achieve in standard well-plate cultures. This versatility makes the platform suitable for a wide range of organoid types, including neuronal organoids for radiation response studies as well as cancer and vascular models for disease-focused applications. The device design allows precise modulation of the biochemical and biomechanical microenvironment through programmable flow and on-chip gradients of oxygen, cytokines, or drugs. With its modular design, compatibility with standard imaging techniques, and capacity for multi-organ co-cultures, the platform is broadly applicable to drug screening, toxicology, personalized medicine, and radiation biology. In summary, the proposed organoids-on-chip system offers a robust and versatile tool for advancing organoid-based research through precise environmental control, real-time monitoring, and physiologically relevant inter-organ communication.

13. Investigating Prenatal Cannabinoid Exposure and Schizophrenia Vulnerability Via Lipidomic Alternations and Impaired Neurodevelopment

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Abstract:
The developing fetal brain is sensitive to environmental perturbations related to drug toxicity, including cannabinoids. Moreover, prenatal cannabinoid exposure (PCE) has been shown to profoundly impair neurodevelopment, leading to disruption of the neurolipidome, neuronal membranes, neurochemical signaling and increased susceptibility to psychiatric disorders, most notably, schizophrenia (SCZ). The mechanism underlying the relationship between PCE and SCZ however, is poorly understood. The endocannabinoid system (ECS) emerges during the first trimester and regulates developmental processes, including neuron proliferation, synaptogenesis and neuroinflammation. Δ9-tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis, is an agonist of the Cannabinoid Receptors 1 and 2 (CB1 and CB2), the canonical receptors of the ECS. Cannabidiol (CBD), also present in cannabis, acts as a negative allosteric modulator of CB1, when present in a high CBD:THC ratio. Thus, cannabinoid exposure may disturb the developing fetal brain. Our research explores the mechanisms behind PCE induced SCZ susceptibility using cerebral organoids derived from human induced pluripotent stem cells (iPSCs). Recent research on prenatal THC exposure has shown neurolipidomic abnormalities in rodents, alongside behavioral deficits resembling prodromal SCZ stages. Species-specific differences in cytoarchitecture and genetic landscape limit current rodent models, as SCZ is a uniquely human disorder involving complex human-specific factors. Therefore, we utilized human-derived cerebral organoids to explore disruptions in the neurolipidome within SCZ and investigated how PCE alters the neurolipidomic landscape. Organoids from healthy control patients (CTRL; n=6) and SCZ patients (SCZ; n=6) were exposed to THC (100nM), cannabidiol (CBD; 500 nM), or THC+CBD combination (100 nM THC+500 nM CBD) or a vehicle group (VEH; no treatment) for 27 days, until organoids reached one month of development, a period resembling early cortical growth. Techniques included lipidomic analyses using matrix assisted laser desorption/ionization-mass spectrometry (MALDI-MS), gene expression assays via immunofluorescence (IF), quantitative PCR (qPCR), and RNA-sequencing (RNA-seq). Preliminary characterization revealed expected neuronal markers in the vehicle-treated Control and SCZ organoids. THC+CBD and THC exposure showed distinct lipidomic and transcriptomic profiles compared to CBD and VEH in control cell lines; this was far more pronounced in the SCZ line. Interestingly, the THC+CBD exposed organoids showed milder impairments compared to the THC treated group, suggesting that CBD can mitigate some effects of THC. The distinct effects of CBD when compared to THC, indicate that these cannabinoids can act independently of each other, as well as concertedly. SCZ organoids exposed to THC expressed alterations in all assessed metrics. These changes resembled preliminary findings of prenatal THC exposure and lipidomic and molecular abnormalities. qPCR showed differential expressions of neuronal markers, particularly in SCZ THC organoids. Comprehensive RNA sequencing data comparing treatment groups will be presented, focusing on lipidomic pathways and phospholipid composition in the neuronal membranes. Immunofluorescence analysis showed alterations in neurodevelopmental, glutamatergic and GABAergic signaling pathways. This study which utilizes cerebral organoids explores cellular-level effects of PCE on brain development and its association with uniquely human psychiatric disorders.

14. Comparison of iPSC-Derived Myeloid Differentiation Protocols Using Single-Cell Mass Cytometry (CyTOF)

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Abstract:
Induced pluripotent stem cells (iPSC) provide a renewable source for generating hematopoietic and stromal lineages for organoid-based tissue development and disease modeling. Recent studies have shown that iPSC-derived $CD14^+$ monocytes/macrophages facilitate organoid maturation and generate tissue-resident macrophage–like populations in co-culture. However, the cellular composition and phenotypic diversity of iPSC-derived myeloid cells generated through hematopoietic differentiation remain incompletely characterized.

Here, we performed single-cell mass cytometry (CyTOF) analysis of iPSC-derived myeloid populations generated from three independent donors using two differentiation protocols: a commercial monocyte differentiation kit (STEMCELL Technologies) and a previously established in-house protocol (Cao et al., Stem Cell Reports, 2019). Cells were harvested and analyzed at day 15 of differentiation across three fractions—adherent cultures (commercial kit only), suspension, and $CD14^+$ magnetic bead–enriched cells to capture the continuum from hematopoietic progenitors to mature myeloid cells.

Unsupervised clustering identified eleven distinct subsets, including core monocyte/macrophage clusters (Cluster 0: $CD33^+CD14^+CD163^+CD169^+$MerTK^hi), a macrophage precursor cluster (Cluster 1), monocytes (Cluster 3: $CD33^+$MerTK^lowCD163^low), and monocyte/DC-like cluster (Cluster 8: $CD33^+CD14^+CD163^+CD169^+$CD11c^lowMerTK^low). Additional subsets represented hematopoietic stem/progenitor cells (Cluster 7: $CD34^+CD90^+CD73^+CD324^+CD141^+CD193^+$), pre-dendritic intermediates (Cluster 9: CD11c^hi$CD33^+CD14^+$), and residual stromal cells (Cluster 2: $CD90^+CD193^+CD73^+CD141^+$).

Pre-sorted adherent cells (commercial kit) were enriched for mesenchymal stromal cells (Cluster 2) and hematopoietic progenitors (Cluster 7). In suspension cultures, the in-house protocol generated higher proportions of early myeloid intermediates including pre-macrophages (Cluster 1) and pre-dendritic cells (Cluster 9), whereas commercial kit yielded higher neutrophil/endothelial cell population (Cluster 6). Following $CD14^+$ selection, both protocols effectively enriched monocyte/macrophage populations (Clusters 0, 1, 3, and 8) achieving approximately 86.7% purity with the in-house and 83.7% with the commercial kit. Residual heterogeneity differed between the two protocols with the in-house protocol retaining more pre-dendritic intermediate cells (Cluster 9) and the commercial protocol maintaining a higher fraction of neutrophil/endothelial cells (Cluster 6).

In summary, both differentiation protocols effectively generate iPSC-derived myeloid cells but differ in lineage composition, purity, and residual progenitor content. This multi-donor, high-resolution CyTOF analysis highlights the need for functional validation to identify the most suitable subsets for specific research and therapeutic applications.

15. Testing Circadian Clock Rhythms in Human Organoids

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Abstract:
Circadian rhythms are internal 24-hour cycles that regulate essential biological processes, such as metabolism, cell proliferation, and immune response. These rhythms are maintained by a central clock in tissues like the liver, intestine, and colon. At the molecular level, the circadian clock operates through a transcription-translation feedback loop involving core clock genes: CLOCK, BMAL1, PER1-3, and CRY1-2. The CLOCK-BMAL1 heterodimer activates the transcription of PERs and CRYs, inhibiting CLOCK-BMAL1 activity, and forming a negative feedback loop. This cycle is tightly regulated by FBXL3, an E3 ubiquitin ligase, which targets CRY for degradation to consolidate rhythmicity. Lifestyle factors such as night shifts, irregular eating, and frequent time-zone travel can disrupt circadian clock feedback cycles, contributing to chronic diseases, including colorectal cancer (CRC). Using intestinal and colon organoids derived from Per2-Luc mice, our lab has previously shown that intestinal organoids sustain free-running circadian clock rhythms for several days in culture, and these rhythms correlate to the expression of target genes of the Wnt and Hippo pathways. To extend these findings to human tissue, we aim to transduce human organoids with luciferase-based circadian clock, Wnt, and Hippo pathway reporters. To further investigate the clock-cancer interactions in human tissue, I will use genetic knockout (KO) organoids of BMAL1 (a non-redundant clock transcription factor), FBXL3 (an E3 ubiquitin ligase that degrades CRY), and APC (the most frequently mutated protein in CRCs). This will determine whether the disruption of these genes alters circadian clock, Wnt, and Hippo transcriptional rhythms. Our objective is to develop methods for studying circadian regulation and tumour-related pathways in human-derived organoids.

16. DNA methylation signatures distinguish pancreatic ductal adenocarcinoma subtypes of patient-derived organoids

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Abstract:
Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) is predicted to become the second leading cause of cancer-related deaths by 2040 with a 5-year survival rate of ~12%. Current therapies include either gemcitabine-abraxane or the poly-agent FOLFIRINOX, which are given based on patient fitness rather than the tumour's molecular characteristics, which does not reflect how well tumours will respond to treatment. Hence, it is necessary to develop patient-specific treatment strategies based on the tumour's molecular characteristics for more accurate outcomes. Transcriptional analysis of PDAC identify "classical" or "basal-like" subtypes which are characterized by the expression of genes involved in pancreatic development or epithelial-mesenchymal transition, respectively. However, while basal-like tumors are more aggressive than classical tumors, this stratification does not correlate to chemotherapy sensitivity. We are examining a model in which rapidly acquired resistance involves epigenetic reprogramming leading to repression of pathways involved in chemosensitivity. Identifying epigenetic profiles that correlate to molecular subtypes or adaptive mechanisms is crucial for the development of tailored regimens. We hypothesize that DNA methylation programs in patient-derived organoids (PDOs) provides a more stable definition of PDAC subtypes and correlates to therapeutic response.

Methods: Genomic DNA was isolated from PDOs derived from the Comprehensive Molecular Characterization of Advanced Pancreatic Ductal Adenocarcinoma for Better Treatment Selection (COMPASS) and Province of Ontario Strategy for Personalized Treatment in Pancreatic Cancer (Prosper-PANC) clinical trials (n=66). Global DNA methylation was assessed using Illumina Human EPIC v2 Methylation array. DNA methylation core signatures were extracted using Non-Negative Matrix Factorization (NMF) and samples clustered using Consensus Clustering to identify DNA methylation-based subtypes. Diffusion trajectory analysis was applied to these profiles to identify evolutionary trajectories of clusters through pseudo-time. Differential methylation analysis was used to further characterize genes differentially methylated for each cluster and assess differential methylation between basal-like and classical subtypes.

Results: Methylation patterns clustered samples into four distinct groups. Unbiased derived clusters did not align with transcriptomic profiles but presented with clinically-relevant characteristics. Trajectory analysis identified two distinct separations characterized by a gain of methylation through pseudo-time space. Differential analysis between basal-like and classical PDOs identified an hypermethylation of classical biomarkers in basal-like PDOs.

Conclusions: PDOs may be a reliable model for targeted therapeutic studies as they maintain patient characteristics. Using an unbiased approach on PDOs, we identified four new distinct methylation profiles with prognostic potential. Similarly, using a differential analysis to identify the methylation profiles of basal-like and classical PDOs, we identified key classical biomarkers are hypermethylated in basal-like tumours. These results support a model in which epigenetic programs are stable from patient profiles to PDOs, and DNA methylation profiling can be used to characterize the epigenetic adaptation mechanisms of PDAC subtypes.

17. Modeling chronic metabolic stress in colonic organoids

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Abstract:
Long-term metabolic stress from high fat diets (HFD) impairs intestinal function and reduces glucagon-like peptide-1 (GLP-1) secretion from enteroendocrine cells. This can lead to disruption of glucose homeostasis and contribute to metabolic disease. Conventional cell lines and acute exposure models fail to capture the complexity of multicellular responses within the intestinal epithelium. However, mouse and human intestinal organoid systems provide a physiologically relevant, tissue-like system to interrogate metabolic stress adaptation in the intestinal epithelium.

To investigate the mechanisms of chronic metabolic stress, we exposed mouse or human colonic organoids to chronic palmitate treatment to induce HFD-like metabolic stress. Butyrate, a bacterial metabolite that supports mitochondrial function and alleviates oxidative stress, was supplemented to determine its protective effects on colonic epithelial cells. The concentration dependence of palmitate on cell viability and GLP-1 releasing enteroendocrine cell function was assessed by the colorimetric MTS assay and GLP-1 secretion assays using a commercially available ELISA kit, respectively. Levels of oxidative and mitochondrial stress were assessed using the fluorescent probe MitoSOX Red, while metabolic shifts toward glycolysis was assessed by measurement of lactate released in culture media. Future assessments of metabolic stress in the GLP-1 releasing enteroendocrine cell population will include generation of a knock-in reporter line with the fluorescent biosensor mito-roGFP under the control of the proglucagon promoter, enabling real-time monitoring of mitochondrial redox changes in live GLP-1 releasing enteroendocrine cells.

Organoid models enable the study of chronic metabolic conditions on epithelial cell function. We have used intestinal organoid models to investigate changes in intestinal epithelial cell function and role of the gut microbiota during pathophysiological states. Our studies will provide valuable insights into colonic physiology, and the therapeutic potential of butyrate to preserve GLP-1 release during obesity and related diseases.

18. Mapping mesenchymal diversity in the developing human intestine and organoids

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Abstract:
The organization of diverse mesenchymal populations during human intestinal development is critical for tissue architecture and function yet remains poorly defined. To construct a comprehensive, tissue-scale map of the developing human small intestine, we leveraged single-cell RNA-sequencing data to build a custom Xenium spatial transcriptomics gene panel covering the diversity of cell types in the human intestine. Analysis was focused on the developing mesenchyme populations (also referred to as fibroblasts or stroma) given the lack of spatiotemporal information about these cell populations. We defined 5 broad mesenchymal populations occupying discrete anatomical locations within the lamina propria and submucosa – the subepithelial cells (SEC), lamina propria fibroblasts (LPF), submucosal fibroblasts (SMF), smooth muscle cells (SMC), and CXCL13+ fibroblasts. Our data reveal dynamic spatial remodeling of fibroblast communities during development and establish molecular markers to distinguish these populations. We leverage this high-resolution atlas to benchmark pluripotent stem cell-derived human intestinal organoids and to demonstrate how this foundational resource can be used to dissect intestinal stromal signaling in a spatial manner, with broad implications for modeling development, regeneration, and disease.

19. Supraphysiologic concentrations of glucose improves kidney organoid differentiation and development

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Abstract:
Background: Organoids are three-dimensional in vitro models that can be generated from pluripotent stem cells to recapitulate aspects of an organ's structure and physiology. Growth conditions and extrinsic factors greatly influence the efficiency and reproducibility of the spatiotemporal patterning of organoid cellular content and structure. Since the first publications of human kidney organoids in 2015, modifications to protocols have occurred, with minimal investigation into their impacts. One noteworthy change is the transition from APEL2 medium to Advanced RPMI 1640 for culture, while some groups have also utilized DMEM medium. One of the key differences between these culture media is the glucose concentration, for which there is a minimal understanding of glucose's role in organoid development.

Objectives: Investigate the differences in kidney organoid development under previously published culture conditions. Additionally, model the effect of the difference in culture medium on kidney organoid development and investigate glucose concentration as a potential driving factor behind the observed differences.

Methods: Using iPSC-derived kidney organoids, differentiation was conducted under defined conditions in APEL2, and maintenance of organoids in three different cell lines was investigated using traditional APEL2 culture medium, Advanced RPMI 1640, and DMEM supplemented with 5 mM glucose to model basal levels. Additionally, DMEM supplemented with 11.1 mM and 14.4 mM glucose to match the concentration of Advanced RPMI 1640 and APEL2, respectively, was investigated to determine if glucose was responsible for the observed changes.

Results: The culture medium was found to have a profound influence on organoid growth rate, with Advanced RPMI 1640 resulting in the highest growth rate. Kidney organoids cultured in Advanced RPMI 1640 showed enhanced tubular cell formation compared to APEL2, while DMEM 5 mM showed declines in growth and development of kidney cell types by immunofluorescence imaging and RT-qPCR. Additionally, it was found that the endothelial and podocyte populations were susceptible to the culture medium. Adding glucose back to DMEM to match the concentrations of APEL2 and Advanced RPMI 1640 resulted in a recovery of the tubule, podocyte, and endothelial cell populations, with the 11.1 mM concentration being found to have the most positive impact on the proximal tubule and podocyte populations, as indicated by marker expression. Metabolic demand was assessed by measuring the glucose utilization of the kidney organoids under each growth condition. Culture in Advanced RPMI 1640 resulted in the most significant change in glucose levels, while the 5 mM basal condition showed a reduction to as low as 1.5 mM.

Conclusions: The investigation of maintenance media conditions suggests that Advanced RPMI 1640 is a suitable alternative to APEL2 for organoid maintenance, with positive impacts on tubule development. Glucose add-back experiments in DMEM suggest glucose concentration plays a key role in modulating kidney organoid development. Our data suggest that basal glucose levels may be insufficient for the maintenance of highly metabolic kidney organoids, and thus metabolic studies using organoids should be calibrated to account for these models' high metabolic demand.

20. Using organoid models to understand patient-specific sensitivity to HDAC5 inhibition

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Abstract:

Introduction: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal cancers diagnosed in Canada. While several cancers have seen an improvement in survival rates, current PDAC therapies result in a dismal 5-year survival rate of ~12%. PDAC is primarily treated with either gemcitabine-based regimens or the combination therapy FOLFIRINOX. However, PDAC is highly resistant to chemotherapy, highlighting the need for novel approaches to increase sensitivity of PDAC cells to these agents. Epigenetic repressors are often over-expressed in PDAC and may be a target for overcoming resistance. While broad spectrum histone deacetylase (HDAC) inhibitors Givinostat and Dacinostat enhance cytotoxic T lymphocyte killing of PDAC cells, toxicity has prevented the widespread use of these inhibitors in clinical trials. Consequently, we suggest more selective HDAC inhibitors may be a better approach in treating PDAC. The type II HDAC, HDAC5, is only expressed in pancreatic pathologies including PDAC. We hypothesize that inhibition of HDAC5-mediated epigenetic repression will increase sensitivity to chemotherapy.

Methods: To determine whether inhibiting HDAC5 increases sensitivity in PDAC, patient-derived organoids (PDOs) derived from PDAC tumours were treated with LMK-235, a highly specific inhibitor for HDAC5, for five days to prevent histone deacetylation. We performed dose dependent analysis to determine the efficacy of LMK-235 inhibition on histone acetylation and cell death across 3 PDOs. LMK-235's effect on HDAC5 function and histone modifications were evaluated by western blot analysis of extracted histones. Dose-dependent sensitivity of PDOs to gemcitabine alone or with optimal doses of LMK-235 was determined over 7 days. Cell viability and growth will be assessed using the Incucyte system and Alamar Blue.

Results: The findings showed that treatment with LMK-235 increased histone acetylation in PDOs compared to controls. Furthermore, the results demonstrated a dose-dependent decrease in cell viability in all PDO lines exposed to various concentrations of LMK-235. Notably, 0.3 µM LMK-235 increased histone acetylation without affecting cell viability. However, at 1 µM, we observed reduced cell viability which may indicate cytotoxicity. In a resistant PDO line, the dose-response curve for the combined treatment of LMK-235 and gemcitabine shifted to the left, and the IC50 was decreased compared to gemcitabine alone.

Discussion: Overall, LMK-235 increased histone acetylation in PDAC PDOs without reducing cell viability. However, PDO-specific responses to LMK-235 were observed that were not related to the molecular subtype and LMK-235 enhanced gemcitabine sensitivity in PDOs. Our findings show how organoids can be used to assess patient-specific sensitivities and provides evidence LMK-235 may improve the treatment of gemcitabine-resistant PDAC.

21. Rspondin 3 promotes Dclk1 positive cell stemness

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Abstract:
Background: Colorectal cancer (CRC) is the 2nd leading cause of cancer death in North America. Inflammatory bowel disease (IBD), characterized by chronic colitis, is a major risk factor for CRC. Despite the link between inflammation and cancer, however, the mechanism by which colitis leads to cancer is unknown. We previously described the Dclk1CreERT2; R26tdTom; APC fl/fl mice model in which APC-mutated Dclk1+ cells become cancer-initiating cells following colitis injury. Moreover, we recently discovered that the R-spondin proteins (RSPO) 1 and 3 are both upregulated during colitis. RSPOs proteins include a family of four secreted glycoproteins (RSPO1–4), and their main function is to activate the Wnt/β-catenin signaling pathway. They exert their action by binding to their receptors LGR4, LGR5 and LGR6. Previous studies suggest that they play a vital role in both tissue homeostasis and regeneration following injury. Thus, to better understand the effects of RSPO1 and 3 on intestinal epithelial cells, we compared the effects of RSPO1 and 3 on APC-mutant Dclk1 positive cells.

Method: We established small intestinal and colonic organoids from our Dclk1CreERT2; R26tdTom; APC fl/fl and Lgr5 DTR; Dclk1CreERT2; R26tdTom; APC fl/fl mice models. After establishment of organoids, we compared the ability of RSPO1 and 3 to induce Dclk1+ cell lineage tracing. Furthermore, we compared the ability of RSPO1 and 3 to generate organoids from a single Dclk1 positive cells.

Results: In both small intestinal and colonic organoids, we observed that RSPO3 induced lineage tracing of Dclk1 positive cells. In contrast, RSPO 1 treatment did not induce Dclk1 positive cell lineage tracing in either the small intestinal or colonic organoids. In a similar way, we observed that RSPO 3 was able to generate more organoids from Dclk1 positive single cells compare to RSPO1. Since RSPO3 preferentially bind to Lgr5 over Lgr4, we wanted to test if RSPO3-Lgr5 interaction was involved in the RSPO3 stemness effect. We previously show that the Lgr5 DTR; Dclk1CreERT2; R26tdTom; APC fl/fl mice expressed 50% less Lgr5 than the Dclk1CreERT2; R26tdTom; APC fl/fl. Interestingly, when we analyze Dclk1+ single cell tracing in the Lgr5 DTR model, we found that RSPO3's effect was lost, while RSPO1 showed results consistent with our Dclk1CreERT2; R26tdTom; APC fl/fl model.

Conclusions: RSPO3, but not RSPO1, is able to induce stemness of the Dclk1 positive cells in our organoid culture. Furthermore, RSPO3 generate twice as many organoids from Dclk1 positive single cells than RSPO1. Based on our data from the Lgr5 DTR mice, the RSPO3 stemness ability seems to be mediated via binding to Lgr5. Although both Rspondin 1 and 3 are upregulated during colitis, our results suggest that Rspondin 3 may be more important in cancer initiation from Dclk1 positive cells.

22. Generation of Patient-Derived Human Liver Organoids from Fresh and Cryopreserved Human Liver Homogenate

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Abstract:
Background: The liver is an essential organ with crucial biological roles in metabolism, detoxification, digestion, and systemic immune homeostasis. Liver disease is a global burden, accounting for 2 million deaths worldwide. Current in vitro experimental models inadequately recapitulate the complex liver microenvironment, limiting our ability to understand and manipulate disease mechanisms for therapeutic development. Three-dimensional liver organoid culture systems can bridge this gap by more accurately modeling hepatic cellular response to genetic and immune perturbations, enabling opportunities for personalized medicine. Previous hepatic organoid protocols rely on fresh total liver homogenate (TLH). The ability to generate organoids from cryopreserved human TLH allows us to leverage archived samples rather than relying on infrequent and unpredictable fresh tissue access. Moreover, utilizing frozen TLH enables the supplementation of organoids with autologous immune cells to study immune-mediated diseases. We hypothesize that organoids can successfully be generated from cryopreserved human tissue with minimal analytical differences to those initiated from fresh TLH.

Methods: Patient-derived organoids were established from TLH obtained from enzymatically perfused caudate lobes from non-diseased neurologically deceased donors or plate-based enzymatically dissociated uninvolved resection tissue from hepatocellular carcinoma patients. After seeding cells in matrigel domes, organoid formation was initiated by addition of culture media defined by Huch et al. (2015). The following metrics were employed to characterize organoids generated from fresh versus cryopreserved human TLH: 1) proliferative rate; 2) confluency; 3) time to initiation; 4) organoid size; 5) duration of culture; 6) cell lineage determination through immunostaining, flow cytometry, and single-cell RNA sequencing; and 7) genetic stability via whole genome sequencing.

Results: Of the 12 samples initiated from fresh human TLH, 11 successfully formed organoids within the first 3 days of plating with 10 of the samples being sustained in culture beyond passage 5. All 6 samples initiated from cryopreserved human TLH successfully formed cystic organoids and were sustained beyond passage 5. From flow cytometry analysis, we observe that organoid initiating EPCAM+ cells comprise 2-3% of TLH. Organoids initiated from fresh human TLH show increases in proliferation, size, and confluency that were correlated with increases in intracellular ATP levels, as a measure of cell viability. Immunofluorescence performed on organoids initiated from fresh human TLH indicates the bipotent nature of the organoids with the coexpression of HNF4a (hepatocytic marker) and CK7 (bile duct epithelial marker) persistent through multiple passages. Immunohistochemistry also indicates the presence of CD45+ immune cells in the body of the organoids.

Conclusion: Hepatic organoids can be successfully generated from both fresh and cryopreserved human TLH and sustained long-term in culture. Through immunostaining, we observe a bipotent liver progenitor cell phenotype along with immune cell (CD45+) infiltration, confirming these organoids as a promising patient-derived model of the liver's complex microenvironment. Future analyses and more samples will permit us to have a better comparison of the aforementioned metrics.

23. Evaluation of novel LKB1 inhibitors as new therapeutics for advanced epithelial ovarian cancer

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Abstract:
Epithelial ovarian cancer (EOC) is a highly aggressive disease, with an 80% relapse rate following surgery and combination chemotherapy, highlighting the urgency for more efficacious therapeutic strategies. EOC is classified into five histotypes, each genetically and phenotypically unique, yet they all follow a common metastatic mechanism. Malignant cells disseminate from the primary ovarian tumor, form spheroids in the peritoneal fluid, then migrate to secondary sites. Previously our lab demonstrated that the stress signaling mediators Liver Kinase B1 (LKB1) and its downstream target AMP-activated protein kinase (AMPK) are crucial for spheroid survival and metastasis. In collaboration with the Ontario Institute for Cancer Research (OICR), we have identified and optimized several potential LKB1-targeting compounds (ASC-069 series and Dinaciclib series) that we have assessed using in vitro kinase and cell-based assays. We propose that direct LKB1 inhibition will reduce spheroid viability and metastasis among all EOC histotypes since spheroids are a universal metastatic mechanism. As a starting point, we have used EOC cell lines representing three histotypes—high-grade serous, low-grade serous, and clear cell carcinoma—to assess LKB1 inhibitor efficacy on spheroid cell viability. We have confirmed varying extents of on-target activity by immunoblotting for phospho-AMPK T172 and plan to assess potential off-target activities, too (i.e., CDK2 substrate Rb protein). Preliminary results indicate that the ASC-069 series exhibits greater potency across multiple cell lines compared to the Dinaciclib series. Current studies are extending from spheroids to recently developed patient-derived organoid (PDO) models of EOC. Given their direct clinical relevance, PDO models will strengthen the rationale for targeting LKB1 as a novel therapeutic strategy in EOC. Assessing drug response in both spheroids and organoids will provide a more comprehensive understanding of how LKB1 inhibition impacts cell viability and disease progression.

24. Human Liver-Derived Organoid Culture for Cellular Reprogramming and Drug Screening for the Treatment of Primary Sclerosing Cholangitis

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4. PSC Partners Seeking a Cure

Abstract:

Background: Primary Sclerosing Cholangitis (PSC) is a rare immune-mediated cholestatic liver disease characterized by biliary tree destruction and progressive fibrosis, leading to end-stage liver disease and transplantation. There are currently no successful treatments for PSC, with 20-30% of patients experiencing disease recurrence post-transplantation. There are no known reliable mouse models, highlighting the need for alternative preclinical approaches to better understand the cellular composition of the PSC liver, to assess potential treatments. Recently, multi-omic characterization of explanted PSC liver tissue (Andrews & Nakib et al. 2024) identified the colocalization of transforming growth factor beta (TGF-B) within fibrotic regions of the PSC liver, highlighting its potential as a therapeutic target for modulating dysfunctional PSC macrophages.

Methods: We have established an organoid model for PSC from cryopreserved total liver homogenates (TLH) of explanted liver samples, with primary biliary cholangitis (PBC) and neurologically deceased donor (NDD) liver samples serving as controls. Organoids were cultured in Matrigel and passaged every 14 days. A selective commercially available TGF-B inhibitor (SB 431542) was introduced into the culture medium at a concentration of 10μM 24 hours post-passage. Culture media was replaced every 3-4 days, and culture supernatants were collected at every media change timepoint. Cytokine bead array (CBA) was utilized to quantify and observe the presence of inflammatory cytokines and chemokines in the culture supernatant. Immunohistochemistry and immunofluorescence were employed to identify retained cell populations across culture and passage timepoints.

Results: We show successful establishment of patient-derived PSC organoids, as well as the presence of CD45+ cells, up to 28 days in culture. IF staining of the organoids revealed the presence of cholangiocytes (CK7+) and hepatocytes (HNF4A+) localized on the lining of PSC organoids, demonstrating their multicellular nature. Further analysis suggested the existence of transitioning hepatocytes by day 14 of culture, as shown by the co-expression of HNF4A andCK7. PSC organoids were significantly smaller in size in comparison to NDD and PBC, consistent with what had been previously described in literature. These findings highlight the potential of organoids as a platform to model PSC, in which access to fresh tissue is limited due to rarity of the disease. TGF-B inhibitor-treated organoids demonstrated a marked increase in size compared by day 10 of culture, in comparison to its untreated counterpart. This suggests evidence of successful antagonism of the TGF-B pathway, resulting in the promotion of organoid growth. Building on this foundation, PSC organoids provide an opportunity to serve as a pre-clinical drug testing platform, where therapies can be evaluated in a patient-specific and multicellular context.

Conclusion: Multicellular PSC organoids were successfully established in this study and were able to recapitulate the complexity of the PSC liver microenvironment. PSC organoids provide an avenue to model complex cholestatic diseases in comparison to single-cell culture, offering a more representative platform for drug screening.

25. A Tumor-Informed Organoid Approach to Personalize ctDNA Detection in Esophageal Adenocarcinoma

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Abstract:
Purpose and Hypothesis: Esophageal adenocarcinoma (EAC) remains one of the most lethal malignancies, largely due to late diagnosis and the lack of reliable biomarkers to detect minimal residual disease or early recurrence. Our project aims to develop a personalized liquid biopsy capable of monitoring treatment response and recurrence by leveraging patient-derived organoids (PDOs) as individualized tumor models. PDOs retain the genetic, epigenetic, and histological features of the parental tumors, providing a clinically relevant platform to study tumor biology and circulating tumor DNA (ctDNA) release. Given the genetic heterogeneity of EAC and the scarcity of recurrent driver mutations, detecting tumor-specific cell-free DNA(cfDNA) is particularly challenging. We hypothesize that PDOs can be used to model ctDNA fragmentation and generate patient-specific mutational and nucleosomal signatures, which can guide the design of highly sensitive ctDNA assays. Since nucleosome positioning influences cfDNA fragmentation, PDO-derived cfDNA can reveal tumor-protected genomic regions that improve detection accuracy and assay specificity.

Methods: PDOs were established from freshly resected EAC tumors and expanded in 3D suspension culture. Chromatin was isolated from PDOs and subjected to micrococcal nuclease (MNase) digestion to obtain mononucleosomes (~147 bp), followed by DNA size selection. MNase-sequencing (MNase-seq) was performed to map nucleosome-protected regions enriched for somatic variants. Matched whole-genome sequencing (WGS) of the patient's tumor served as a reference to validate the PDO-derived mutation landscape. Primers targeting the identified patient-specific mutations were designed and used to amplify DNAfrom PDOs and plasma cfDNA samples.

Results: DNA from five independent EAC PDO lines was successfully digested with MNase, yielding highly enriched mononucleosomal fragments. MNase-seq identified 24 somatic mutations within nucleosome-protected regions across 24 genes, including known oncogenic drivers. PCR and targeted sequencing confirmed the detectability of six of these genes in both PDO-derived cfDNA and control cfDNA samples. Using dilution series (0.01%–100%) with reference materials, we demonstrated the feasibility of detecting low-frequency mutant alleles, validating the sensitivity of our approach. The PDO-derived cfDNA faithfully recapitulated the fragmentation and mutational profiles observed in matched patient plasma, underscoring its physiological relevance.

Conclusions: Our findings demonstrate that patient-derived organoids provide a robust and translational model to decode ctDNA biology and personalize liquid biopsy design in EAC. PDOs not only mirror the tumor's mutational landscape but also recapitulate the chromatin-driven cfDNA fragmentation patterns that shape ctDNA detection. By using PDOs as a preclinical tool, we can generate a patient-specific nucleosome mutation map to guide personalized PCR-based assays for monitoring residual disease and predicting recurrence. This strategy highlights the potential of PDOs to bridge molecular oncology and precision medicine, offering a pathway to earlier detection and tailored therapeutic management in esophageal cancer.

Presenters Poster Session II

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Bianca Garlisi University of Guelph	Investigating PDAC Murine Organoids' Cell Viability with the Treatment of the Novel Therapy Fc3TSR	D
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Domenic Di Stasi Western University	Defining cancer cell and fibroblast interactions using patient-derived organoids in pancreatic cancer	G
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Wessam Al Jawhri Western University	Time-resolved genomic, transcriptomic, and microenvironmental landscape during HPV-induced carcinogenesis.	X

A. Development of hPSC-derived Multi-Lineage Liver Organoids Incorporating Hepatic Stellate Cells for Hepatic Maturation

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Abstract:
The liver's intricate architecture and diverse functions depend on dynamic interactions between hepatocytes and non-parenchymal cells (NPCs). In their absence, liver organoids composed solely of hepatocytes lack physiological relevance and fail to achieve full functional maturation. Among NPCs, quiescent hepatic stellate cells (qHSCs) are liver-specific mesenchymal cells that regulate extracellular matrix (ECM) homeostasis and store vitamin A as retinyl esters. Building on this understanding, we developed a protocol to generate $WT1^+$ septum transversum mesenchyme (STM) progenitors—precursors to HSCs—from human pluripotent stem cells (hPSCs). These STM-derived cells were co-cultured with hPSC-derived ALB^+/AFP^+ hepatoblasts (HBs) to form hepato-STM organoids (HSOs), enabling investigation of cell-cell interactions and the functional contributions of mesenchymal support to hepatic maturation.

HBs and $WT1^+$ STM-like cells were co-cultured at a 3:1 ratio for 12 days, forming self-organizing 3D spheroids with STM cells enveloping the HB core. FACS and RT-qPCR revealed significant upregulation of lecithin retinol acyltransferase (LRAT) and nerve growth factor receptor (NGFR) in STM cells from HSOs versus monocultures, indicating enhanced quiescence and differentiation toward mature HSC phenotypes in the presence of hepatic cells. Upon retinol and palmitic acid treatment, flow cytometry revealed intracellular autofluorescence (AF)—an indicator for retinol storage—in 64.50% ± 6.31 of STM cells in HSOs, whereas AF was nearly absent in untreated conditions (1.44% ± 0.55). STM cells in HSOs exhibited reduced AF relative to monocultures, while HB cells showed increased AF, suggesting retinoid redistribution from stellate cells to HBs within the organoid. Collectively, these findings indicate that STM cells within HSOs acquire quiescent, HSC-like characteristics in a HB-dependent manner.

To assess the impact of mesodermal identity on HB fate, we compared HSOs with hepato-mesenchymal organoids (HMOs), which were formed using hPSC-derived mesenchymal stem cells (MSCs)—a widely utilized cell type in conventional liver organoid models. Unlike STM-derived cells, MSCs failed to acquire hepatic stellate cell (HSC)-like features, exhibiting low autofluorescence (AF) and minimal LRAT expression, both of which were robustly induced in STM-derived populations. Notably, the frequency of $CK7^+$cholangiocyte-lineage cells was significantly lower in HSOs (23.73% ± 2.11) than in HB-only (67.83% ± 14.20) and HMO(66.63% ± 10.26) conditions, suggesting that STM-derived cells suppress cholangiocyte lineage commitment and potentially bias HBs toward hepatocyte differentiation.
To evaluate the impact of mesenchymal maturity on hepatocyte function, we substituted STM progenitors with more differentiated HSCs to generate hepato-HSC organoids (HHOs). HHOs exhibited significantly higher retinol retention compared to HSOs, with levels comparable to those observed in monolayer-cultured HSCs prior to co-culture—confirming the maintenance of quiescent, functionally active HSCs within the organoid. CYP3A4 activity assays revealed significantly higher hepatocyte metabolic function in HHOs than HSOs, suggesting that differentiated HSCs provide a more supportive microenvironment for hepatocyte maturation than STM progenitors.

Taken together, our HSO and HHO organoid platforms offer a robust and versatile system for modeling liver pathologies, particularly those involving hepatic stellate cell (HSC) activation and fibrogenesis. By leveraging the modularity of our organoids, we enable disease stage-specific therapeutic testing and patient-specific drug screening using iPSC-derived components.

B. BMP Signaling Promotes Immune Cell Precursor and Enteroendocrine Cell Differentiation in Human Intestinal Organoids

Abigail Vallie, Charlie Childs, Xiangning Dong, Sha Huang, Yuhwai Tsai, Angeline Wu, and Jason Spence

University of Michigan

Abstract:
Background: Pluripotent stem cell-derived (PSC) human intestinal organoids (HIOs) are a complex in vitro model system that recapitulates some aspects of the intestinal architecture and function and can be used to model intestinal physiology and pathophysiology. Recently our lab has shown that culture conditions and growth factors influence the cellular composition of HIOs, which can be induced to include epithelial, mesenchymal, endothelial, neuronal, serosal and smooth muscle cells through differentiation of pre-existing populations of mesoderm and endoderm in the early HIO culture. Despite these incredible advances, HIOs have still lacked robust populations of immune cells. The Wells lab (Múnera et al.) showed that BMP is important for patterning the human intestine into the colonic region and can also induce immune cells within human colonic organoids (HCOs). Thus, the dual role for BMP signaling in immune cell induction, and its role in distal (colonic) intestinal patterning presents a challenge for generating proximal (small intestinal) HIOs that possess immune cells. That is, BMP is required for immune cell induction, while inhibition of BMP via NOGGIN is required for patterning into a small intestinal fate.

Method(s): Differential gene expression analysis was carried out on previously published single cell RNA sequencing (scRNAseq) data, to identify cell types present in early 3D HIO spheroids. HIOs were generated using previously established methods with the addition of 50ng/ml BMP after intestinal patterning to determine if immune cell populations could be induced. RT-qPCR was performed to examine expression of PTPRC (CD45), a marker used to evaluate the presence of immune cells in culture, PECAM1, CD34, RUNX1, and SPN (CD43) indicative of immune cell precursors, and ATOH1, SPEDEF, and CHGA which mark the enteroendocrine cell lineage. Additionally, HIOs were stained using immunofluorescence techniques to identify immune cells via CD45 expression, immune precursors via PECAM1, CD34, RUNX1, and CD43, and epithelial cells via ECAD, enteroendocrine cells via CHGA expression, and goblet cells via MUC2.

Results: scRNAseq of HIO spheroids showed that early 3D HIO spheroids are composed of primitive CDX2+ epithelium and undifferentiated mesoderm, suggesting that mesoderm at the spheroid stage may respond to hematopoietic inductive cues. Based on prior knowledge and our new findings, I developed a multi-step induction method, where HIOs are patterned into small intestine, and treated with BMP4 to induce immune-like cells (which are not present during normal HIO differentiation). I show that this stepwise method induces co-expression of CD34 and RUNX1, and CD43 positive cells, indicating immune precursor cells, such as hematopoietic stem-like cells and hemogenic endothelium-like cells. Unexpectedly, BMP-treated HIOs also showed a dose-dependent increase in enteroendocrine cells and goblet cells, marked by CHGA and MUC2.

Conclusions: BMP4 treatment is sufficient to fundamentally change the mesenchymal and epithelial cell complexity in HIOs. Increased expression of markers for immune cell precursors indicates a role for BMP signaling in mesoderm differentiation. Moving forward, I will test the hypothesis that undifferentiated mesoderm progenitors are maintained for several days during the early spheroid phase of the HIO and can be directed into many different tissue lineages including immune cells and their precursors. Furthermore, the increase in secretory cells, including CHGA+ enteroendocrine cells after BMP4 treatment suggests that BMP signaling may play a novel inductive role in the secretory lineage, a finding that will be the focus of future research. This work begins to develop a method for creating a more complete, immunologically-competent HIO, while also testing the plasticity of mesoderm progenitors in the HIOs, and the role of BMP signaling in enteroendocrine cell differentiation.

C. Identifying microRNA markers of embryo developmental potential in human pluripotent stem cell-derived blastoids

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3 Children's Health Research Institute, London, ON, Canada

Abstract:
With rising trends in delayed childbearing, in vitro fertilization (IVF) has become a vital assisted reproductive technology worldwide. However, its success rates remain low, primarily due to aneuploidy—an abnormal chromosomal gain or loss that can impair development. Current embryo selection methods are subjective and invasive, limiting their effectiveness in determining ploidy status.

We aim to develop a quantitative, non-invasive assay that accurately assesses an embryo's developmental potential while preserving its integrity, using microRNAs (miRNAs)—small, non-coding RNAs secreted by embryos into their surroundings. Supporting their role in regulating gene expression and implantation, specific miRNAs have been identified as potential biomarkers, differentially expressed in the media of failed or low-morphological-grade IVF embryos.
While promising, studying the miRNA profiles of human embryos is challenging due to ethical and logistical limitations. Blastoids—embryo-like structures derived from human embryonic stem cells (hESCs)—offer an accessible, ethical alternative for modelling human preimplantation development. However, it remains unclear whether blastoids exhibit development-specific miRNA patterns that are representative of those of human embryos.

To investigate this, we cultured naïve-like H9 hESCs for five days to generate euploid, vehicle control, and aneuploid blastoids. Aneuploidy, our proxy for low potential embryos, was induced using AZ3146—a spindle assembly checkpoint inhibitor. Immunofluorescence was used to characterize the model, including confirmation of ploidy status through centromere detection and analysis of primary cell lineage markers. Using RT-qPCR and Droplet Digital PCR, we will quantify twelve candidate miRNAs selected from the literature in blastoids and spent media, then validate the observed trends with clinical media samples. Uncovering ploidy-specific miRNA profiles could enhance embryo selection in IVF, facilitating improved clinical outcomes and patient quality of care.

D. Investigating PDAC Murine Organoids' Cell Viability with the Treatment of the Novel Therapy Fc3TSR

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Abstract:
Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) has a poor survival rate due to late diagnosis where metastasis has often occurred. Angiogenesis, the process by which new vessels form from pre-existing vasculature, is crucial for tumour growth and metastasis. Tumours aggressively upregulate expression of pro-angiogenic factors, stimulating rapid vessel formation. These vessels often lack pericyte coverage and have dysfunctional morphology, reducing perfusion and contributing to high interstitial fluid pressure (IFP). This vascular dysfunction can impede therapy uptake inhibit immune cell migration to the tumour and tumour draining lymph nodes. Fc3TSR is a novel fusion protein derived from the type I repeat region of the endogenous angiogenic inhibitor thrombospondin 1, which we have shown in 2D cell culture to cause apoptosis, reduce proliferation, and reduce invasion and migration showing promising results. While 2D cell culture provides for many simple yet critical experiments in advanced diseases like PDAC, they unfortunately lack important cell to cell interactions, which can reduce translational impact. Organoids offer a 3D representation of the tumour, more accurately accounting for the physiological interactions and growth of tumour cells. In addition, organoids can be directly formed from mouse or human tumours, maintaining the heterogeneity found within tumours. Here, we optimized PDO experiments to evaluate the effect of Fc3TSR on PDAC murine organoids viability and determine varying dosage time and concentration effects.

Methods: We obtained numerous murine PDAC organoids from Dr. Chris Pin's lab at the University of Western Ontario. We cultured 4 of the organoid lines in previously established culture medium before seeding them in 10uL Matrigel domes in a 96 well plate at cell densities of 3000 cells. Organoids were allowed to grow for 24 hours, before being exposed to treatments of Fc3TSR at 0, 250, 500, 750, 1000, 1250, and 1500nM for 24 or 48 hours. At the 18 or 24 hour mark respectively, alamarBlue HS Cell Viability Reagent was added to the treatment mixture and allowed to stain the organoids. The plate was read at an absorbance of 630nM and the metabolic activity (representative of viability) was analyzed.

Results: With only 6 hours of alamarBlue staining, there was relatively inconsistent results compared to our 24 hour staining, which we then used going forward. Increasing concentration of Fc3TSR decreased viability in our EF5 organoid line, with metabolic activity decreasing to 35% at 500nM. We then saw a rebound in metabolic activity to 50% as concentrations increased to 1000nM, which then dropped again to 10% at the maximum dose of 1500nM. The EE2 organoids had a similar trend as the EF5 organoids, with an increased activity at the 250nM treatment compared to our untreated control, decrease to about 60% activity until the 750nM concentration, before rebounding to 80% at the 1250nM concentration. The EA2 organoids activity increased with the 250nM treatment compared to the untreated control, and steadily decreased until the 1250nM treatment before having a slight rebound at 1500nM. These preliminary results suggest that Fc3TSR reduces viability of murine PDAC organoids, although further experiments are underway to confirm this.

Discussion: While 2D culture can offer many answers in PDAC disease progression and treatment intervention, these results are often not translated when moving past cell culture. With increased alamarBlue staining of 24 compared to 6 hours, it seems the dye was able to thoroughly and consistently penetrate the Matrigel domes and stain the organoids. These preliminary organoid experiments suggest that while Fc3TSR still has an effect on PDAC cells, it is not as simply explained in regards to our 2D cell culture finding, with much variability between organoid lines.

E. Identification and functional characterization of the cancer-initiating cell population in human ovarian clear cell carcinoma (OCCC) lines

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Abstract:
Introduction: OCCC is a rare histotype of epithelial ovarian cancer with a poor prognosis if diagnosed at a late stage. One determinant of OCCC mortality is the extent of regional and distant metastases within the peritoneal cavity. Metastasis is responsible for 90% of cancer deaths and can be attributed to a small subpopulation of highly tumorigenic cells with a capacity for self-renewal, known as cancer stem cells (CSCs). Despite making up 0.01-2% of the average tumour mass, these cells play a crucial role in treatment resistance, disease spread, and recurrence.

Methods: RT-qPCR, Western blotting, and FLOW cytometry were employed in OCCC cell lines to establish a baseline expression profile for well-characterized CSC markers. Select lines were used for CSC enrichment through the development of drug-resistant lines or through specialized culture conditions meant to promote stem-ness. Changes in the expression of well-characterized CSC markers in enriched lines will be investigated relative to the pre-enrichment parental cell line and will be transcriptionally characterized via bulk RNAseq.

Results: The expression of different stem cell markers (NANOG, SOX2, c-MYC, KLF4) did not correlated with the expression of ALDH1A1, nor the activity of ALDH family members, in human OCCC cell lines. Ability to proliferate in suspension culture in standard media did not predict a cell lines viability in suspension culture using CSC enriching media. Despite being a bona fide pan-cancer marker of CSCs, ALDH1A1 protein expression is decreased or completely lost in CSC spheres. While short term drug treatment appears to promote a stem-like phenotype in adherent cells, the development of drug resistance to paclitaxel and AZD-8055 did not result in a more stem-like phenotype in resistant spheroids.

Conclusions: This is the first time human OCCC cell lines have been enriched with CSCs for identification and functional characterization. These results may provide a platform for future studies aimed at understanding how various epigenetic features define an OCCC CSC from a bulk tumour cell and thus potentially identify existing epigenome-targeted therapies which may selectively kill CSCs.

F. Characterizing p66Shc-dependent effects on pluripotent stem cell fate using teratomas and single-cell transcriptomics for comparative in vivo multi-lineage differentiation modelling

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Abstract:
When stem cell regulation is disrupted during early ontogenesis, developmental process timing and synchrony defects often elicit maturational delay and arrest. As key drivers of stem cell fate and state decisions, mitochondrial homeostasis, metabolism, and redox state are interconnected through the p66Shc adaptor protein. Our lab previously induced p66Shc knockout (KO) in R1 mouse embryonic stem cells (mESCs) and demonstrated that acclimation to defined feeder-free cell culture (2i/LIF) abrogated phenotypic differences with wildtype (WT) mESCs in the naïve pluripotency state; the resulting murine allograft teratomas exhibited immature development. Like organoids, teratomas enable developmental potential modeling; their vascularized 3D environment and regions of complex tissue-like organization continue to inspire exciting innovations, including vascularized organoids. However, heterogeneity poses challenges for distinguishing p66Shc-dependent effects across distinct cell types. Here, advances in single-cell RNA sequencing show promise. In this study, we acclimated a WT and two p66ShcKO mESC clones to 2i/LIF conditions from which we generated teratomas in female NOD/SCID IL-2Rγ-null mice. Alongside cells of all three germ layers in WT (n = 11) and p66ShcKO (n = 13) teratomas, histopathological analysis revealed uniform putative early-developmental-stage cell populations enriched in the latter, which were associated with well-differentiated structures in WT. To capture intra-tumour heterogeneity, we validated a nuclei isolation and fixation protocol from OCT-embedded tissue that incorporates sections across intra-tumour regions, yielding 70,000 nuclei/mg, complemented by parallel serial histological assessment of these same regions. After rigorous quality control, 303,613 nuclei from eight teratomas were profiled. Of these, a preliminary filtered dataset of definitively-Y-chromosome-gene expressing nuclei shows clear Gene Ontology enrichment (log10P = –17.66) of mechanisms associated with pluripotency in p66ShcKO teratomas. After forthcoming improvements to our Y-chromosome gene scoring model to expand our subset of confidently-mESC-derived nuclei, we will characterize cell types and construct differentiation trajectories. This research aims to provide a robust and representative evaluation of p66ShcKO-induced effects on developmental cell fate decisions, thereby helping to elucidate the mechanisms that underpin mitochondrial control of this fundamental process.

G. Defining cancer cell and fibroblast interactions using patient-derived organoids in pancreatic cancer

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Abstract:
Pancreatic cancer is the 3rd leading cause of cancer-related deaths, with a 5-year survival less than 13%. A major reason for this dismal survival rate is the tumour microenvironment (TME), which comprises up to 90% of the tumour in some cases. The TME consists of a dense stroma that includes cancer-associated fibroblasts (CAFs), immune cells such as tumour-associated macrophages, and endothelial cells amongst other cell types. The TME shows significant inter-patient heterogeneity which contributes to a range of cancer cell phenotypes and therapeutic resistance. Moreover, spatial transcriptomics confirms regional differences for both cancer and non-cancer cells within individual tumours. For example, myofibroblastic (my) CAFs are closely associated with tumour cells while inflammatory (i) CAFs are often located away from tumour cells. While several studies have shown CAFs affect pancreatic ductal adenocarcinoma (PDAC) progression and therapeutic resistance, the mechanisms by which CAFs promote these differences is still unknown. We hypothesized that CAFs secrete factors that promote epigenetic reprogramming that underlies the heterogeneity and resistance observed in PDAC.

To address this hypothesis, we utilized our living biobank of patient-derived organoids and cancer associated fibroblasts obtained from endoscopic ultrasound (EUS) biopsies. To determine how the CAF subtypes affect tumour cell phenotypes and gene expression, we assessed the expression of markers linked to classical (CLDN18, GATA4) or basal-like (ANXA8, KRT17) PDAC molecular subtypes. Marker expression in cancer cells was examined both immediately after isolation from EUS samples and following subsequent culture, either in co-culture with CAFs or after selection and growth without CAFs. Additionally, we treated several patient-derived organoid (PDO) lines with conditioned media derived from CAF cultures enriched for myCAFs or iCAFs. We determined enrichment based on flow cytometry and RNA-seq analysis. 7 days after treatment with conditioned media, the histological phenotypes, proliferation and gene expression profiles were examined.

Preliminary data indicate PDAC cancer cells shift their phenotype when exposed to CAFs or conditioned media derived from CAFs. The presence of CAFs allows for differential growth and conditioned media from both iCAF and myCAF enriched samples appears to increase proliferation in PDAC cells. More specifically, iCAF-enriched media promotes a more basal-like phenotype based on phenotype and the expression of KRT17. These changes appear to be mediated by epigenetic events as they remain after conditioned media has been removed with ATAC-seq showing reduced chromatin accessibility in response to CAF-conditioned media. Additional experiments will be needed to elucidate the identity of the factors secreted by CAFs that promote these changes.

These experiments suggest CAFs promote or maintain molecular subtypes in PDAC, indicating a possible therapeutic advantage in targeting CAFs to promote a less aggressive phenotype. Future avenues of research will target metabolites and epigenetic regulators that may be involved in this cross-talk.

H. Dissecting Morphological Heterogeneity in Pancreatic Cancer Identifies Distinct Basal Cell States via Multi-Omic and PDO Analysis

Eugenia Flores-Figueroa, Yuanchang Fang, Maryam Monajemzadeh, Ayah Elqaderi, Tom Ouellette, Amy Zhang, Gun Ho Jang, Karen Ng, Milena Gallucci, Zhen-Mei Liu, Nikolina Radulovich, Nhu An Pham, Anna Dodd, Julie Wilson, Erica S. Tsang, Steven Gallinger, Jennifer J. Knox, Grainne M. O'Kane, Faiyaz Notta, Robert C. Grant

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Abstract:

Background:

Histological evaluation remains central to the diagnosis, stratification, and prognosis of pancreatic ductal adenocarcinoma (PDAC). Tumour morphology broadly distinguishes glandular from non-glandular patterns, corresponding to classical and basal transcriptomic subtypes, respectively. However, this dichotomous classification fails to capture the morphological continuum that underlies disease progression.

Methods:

We implemented a histology-guided multi-omic strategy integrating transcriptomic and genomic profiling across a curated clinical cohort encompassing all stages of PDAC. The cohort consisted of 319 patients, including 41% with resectable tumours (Stages I–II), 7% with locally advanced disease (Stage III), and 52% with distal metastases (Stage IV). To evaluate whether patient morphology and molecular states can be modeled experimentally, we also analyzed 163 patient-derived organoids (PDOs) established from matched clinical specimens spanning all disease stages.

Results:

Tumours from early-stage (I/II) disease were predominantly glandular, whereas advanced-stage cases exhibited increasing proportions of non-glandular morphologies. To capture this heterogeneity, we subdivided non-glandular tumours into cribriform, solid, and squamous subtypes. Each displayed distinct molecular and phenotypic characteristics, supporting their classification as separate biological entities. Cribriform tumours represented an intermediate morphological and transcriptomic state, characterized by enrichment of partial epithelial-to-mesenchymal transition (pEMT) signatures and reduced classical epithelial programs. These features suggest a plastic population capable of progression toward solid–squamous states or reversion to glandular morphology. Solid tumours displayed strong basal and EMT signatures, while squamous tumours exhibited keratinization and squamous differentiation. PDO analyses confirmed that the morphological and transcriptomic heterogeneity observed in patients can be recapitulated in vitro, reinforcing the biological relevance of these intermediate states.

Conclusions:

Our study demonstrates that histology-guided multi-omic profiling reveals distinct basal programs underpinning the morphological spectrum of PDAC. Recognizing and classifying intermediate and basal morphologies enhances our understanding of tumour evolution and provides a refined framework for linking histology to underlying biology and potential therapeutic vulnerabilities. These findings underscore the clinical relevance of morphology as a molecular and prognostic surrogate in pancreatic cancer and establish PDOs as functional models to study morphological plasticity.

I. Millimeter-Scale Tumoroids Recapitulate In Vivo Tumor Architecture and Enhance Prediction of Complex Drug Responses

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Abstract:
The promise of personalized oncology is fundamentally limited by preclinical models that fail to accurately predict patient-specific drug responses. While patient-derived tumoroids are promising in vitro models, their conventional microscale dimensions do not recapitulate the complex tumor architecture, such as intratumoral heterogeneity, necrotic cores, and physiologic hypoxia, that drives therapeutic resistance in solid tumors and critically governs therapeutic outcomes in vivo.

In this study, we address this critical limitation. Leveraging our recently developed ReSCUE microfluidic platform, we demonstrate the rapid and scalable generation of millimeter-scale breast cancer tumoroids. These large-scale models, containing over one million cells and formed within four days, faithfully replicate key hallmarks of their parent tumors, including the development of a hypoxic core and the preservation of cellular heterogeneity. This advance provides a high-fidelity tumor model for rigorous therapeutic evaluation.

The key advantage of these millimeter-scale tumoroids is their amenability to in-depth downstream analysis. We show that a single tumoroid can be sectioned for multiplexed biomarker profiling, enabling a detailed and spatially resolved assessment of drug efficacy and mechanisms of action. Applying this capability, we evaluated patient-specific responses to a panel of targeted therapies, including antibody-drug conjugates and small molecule inhibitors. The resulting data demonstrated that our large-scale tumoroids predict clinical drug responses with an accuracy comparable to that of patient-derived xenograft (PDX) models but achieved in a fraction of the time.

By capturing the architectural and cellular complexity of solid tumors, this platform offers a more faithful in vitro model at a speed and scale amenable to clinical timelines. This work provides a robust methodology to accelerate the development of effective, personalized cancer treatments, bridging the gap between bench-side discovery and patient-side reality.

J. Endothelial Dysfunction in Loeys-Dietz Syndrome: Insights from Patient Tissue and development of a hiPSC-derived Microfluidic Model

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Abstract:
Introduction: Loeys-Dietz syndrome type 3 (LDS3) is a genetic disorder characterized by aggressive aortic aneurysms amongst other vascular and skeletal pathologies. It is caused by loss of function mutations to SMAD3, a transcription factor responsible for driving transforming growth factor beta (TGF-β) signalling. While LDS3 has been characterized as a vascular smooth muscle cell (VSMC)– driven condition, endothelial cells (EC) are also be regulated through TGF-β signalling and play an important role in aortic homeostasis. Currently, induced pluripotent stem cell (iPSC)-derived endothelial cells (iECs) are of great interest for patient-specific disease modelling, and 3D vascularized complex in vitro models are gaining popularity as they integrate mechanosensory and environmental cues. Thus, the goals of this study are two-fold: first, to determine whether endothelial cells contribute to vascular fragility in LDS3; and second, to optimize a microfluidic model for studying iEC function for future use investigating LDS3 iEC functional changes.

Methods: Aortic wall samples from 6 LDS3 patients and 9 heart transplant patients were collected and sectioned. Sections were DAB stained for podocalyxin, an endothelial marker, and the density of medial vessels was determined. Aorta sections were also stained using H&E and Movat's Pentachrome to interrogate changes in cell morphology and aortic structure. To generate a microfluidic model, iPSCs were differentiated into endothelial cells (iECs), which were seeded into a microfluidic channel (80µm x 80µm x 1cm) and subjected to flow (1µL/min) for either 72 or 120 hours. Cells were then fixed and immune stained assess morphology.

Results: Aorta sections of LDS3 patients showed a reduction in vasa vasorum in comparison to heart transplant patients. Additionally, a fragmented internal elastic lamella, mis-oriented smooth muscle cells, an increase in infiltrating immune cells, and a poorly defined intima-media boundary could be seen in several of the LDS3 patients. In the microfluidic device, changes in iEC morphology, including shear response, could be successfully interrogated. Changes in cell surface areas, aspect rations, alignment of cell body, and actin organization could be seen between 72 and 120 hours as cells responded to flow.

Conclusion: The LDS3 patients show intimal and vasa-vasorum changes, which could be indicative of altered EC function. Microfluidic modelling was able to capture subtle changes in hiEC morphology, suggesting the model will be able to capture functional and morphological differences when using LDS3-affected iECs.

K. Knockout of Trp53 suppresses stemness of cancer-initiating cells

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Abstract:

Introduction

Colorectal cancer is the second leading cause of cancer-related death, with chronic inflammation being a major risk factor. We previously described a colitis-associated cancer (CAC) model in which tumors arise from Dclk1+ cells following APC loss and colitis induction. In human CAC, mutations in TP53 are often detected in early dysplasia. Recent studies have also shown that acute p53 loss can induce the expression of transposable elements (TEs), viral elements in the DNA that can activate a type-I interferon response via the cGAS/STING and MDA5/MAVS pathways, a response known as viral mimicry. We have recently demonstrated that activation of viral mimicry through DNA hypomethylation can suppress stemness of cancer-initiating cells. Thus, we hypothesize that p53 loss induces viral mimicry, thereby inhibiting the stemness of cancer-initiating Dclk1+ cells.

Methods

Colonic organoids were derived from Dclk1/TdTomato/Trp53f/f, Dclk1/TdTomato/Apcf/f and Dclk1/TdTomato/Apcf/f/Trp53f/f mice. Following organoid establishment, 4-hydroxytamoxifen (4-OH-TAM) was added to the cultures in order to label Dclk1+ cells and knockout Apc and/or Trp53. Following 4-OH-TAM treatment, organoids were dissociated into single cells. The number of TdTomato+ cells were then quantified on day 1 and the number of TdTomato+ lineage traced organoids quantified on day 7 to determine the organoid-forming ability of Dclk1+ cells. To assess whether the differences in stemness were due to a viral mimicry response, colonic organoids from Dclk1/TdTomato/Trp53f/f, Dclk1/TdTomato/Apcf/f and Dclk1/TdTomato/Apcf/f/Trp53f/f mice were treated with 1uM cGAS inhibitor for 48 hours and the organoid forming ability assessed. Additionally, we knocked out MAVS in the same organoids to further investigate the role of the viral mimicry response.

Results

APC loss induced organoid forming ability in 5-10% of Dclk1+ cells. p53 loss significantly inhibited organoid formation and thus stemness of Dclk1+ cells, both in the presence or absence of APC loss. Interestingly, treatment with a cGAS inhibitor rescued the loss of stemness and enhanced organoids formation of Dclk1+ cells lacking both APC andp53. Similarly, MAVS knockout reversed the inhibitory effect of p53 loss on stemness, suggesting that activation of the cGAS/STING and MDA5/MAVS pathways mediate the effects on stemness.

Discussion

Our findings demonstrate that p53 loss suppresses stemness of APC mutant Dclk1+ cells through activation of the cGAS/STING and the MDA5/MAVS pathways. This highlights a novel mechanism by which p53 loss may exert tumor-suppressive effects in the early stages of colitis-associated cancer.

L. Infrastructure to Enable Collaborative Discovery and Model Identification at the Princess Margaret Living Biobank Organoid Core

Guanqiao Feng, Quan Li, Ming Tsao, Nikolina Radulovich

Abstract:
Organoids provide a patient-relevant and efficient platform for pharmacogenomic discovery, combining the biological flidelity of patient-derived xenografts (PDXs) with the scalability of cell lines. The Princess Margaret Living Biobank (PMLB) Organoid Core offers high-quality organoid models and comprehensive support services to accelerate discoveries in both academic and industry settings.

Our collection currently includes over 400 organoid models derived from diverse tumour types, including lung, pancreatic, colorectal, ovarian, esophageal, breast, mesothelioma, cholangiocarcinoma, and endometrial cancers. To ensure these models are established and utilized with consistency and reliability, the PMLB Organoid Core implements standardized protocols and stringent quality control measures, including pathology assay, histology assay, growth assay, flow assay, Short Tandem Repeat (STR) and Single Nucleotide Polymorphism (SNP) tests. To enhance accessibility and foster collaboration, we are developing an integrated infrastructure that includes the PMLB handbook—a public website providing up-to-date documentation of model databases and standard operating procedures (SOPs); cBioPortal—an interactive platform for clinical and multi-omic data exploration; GitHub repositories—hosting bioinformatics workflows and analysis pipelines; CDD Vault—a centralized database for drug screening data; and a Chatbot—providing user-friendly access to clinical and molecular data.

Together, these interconnected resources promote transparency, streamline data access, and empower collaborators to efficiently identify and select organoid models relevant to their research.

M. Enhancer-Promoter Communication in Neurodevelopmental Disorders Modeled with Human Brain Organoids

Liu X, Ayekun L, Iturralde B, Gloade A and **Tobias IC**

Abstract:
Mutations affecting chromatin regulatory proteins disrupt brain development and contribute to neurodevelopmental disorders (NDDs) ranging from autism spectrum disorder to intellectual disability syndromes. A proposed pathogenic mechanism common to multiple NDDs is that deficient or dysfunctional chromatin regulatory proteins impair the fidelity of molecular communication between distal regulatory elements, such as enhancers, and their target gene promoters. However, the specific principles of three-dimensional genome organization that make certain genes susceptible to enhancer mis-targeting and altered neurodevelopmental gene expression programs remain poorly understood.

The overall theme of my lab is to understand how enhancers and insulators operate within 3D chromatin architecture to direct gene expression and how perturbations in these interactions drive neurodevelopmental pathology. We model chromatin regulatory protein defects using CRISPR/Cas9-engineered human iPSCs and brain organoids, focusing on dosage-sensitive proteins that control the function of enhancers and topologically associating domain (TAD) boundaries.

To understand the developmental consequences of these genetic manipulations, we use two complementary approaches: (1) genome-wide chromatin accessibility and conformation assays to characterize key nodes in the cis-regulatory landscape active in purified neural progenitor cell systems, and (2) bulk and single-cell transcriptomics to resolve temporal and cell type-specific developmental processes in brain organoids. We propose a set of experiments to identify mechanisms of enhancer-promoter dysregulation across distinct NDD models and to discover chromatin regulatory pathways amenable to gene-editing and epigenetic therapies.

N. Interrogating Diverse Injury Response Programs in the Damaged Human Intestine Across Different Injuries

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Abstract:
Background: The small intestine serves a dual role in the body by absorbing nutrients and functioning as a protective barrier against external stressors. Given the intestinal epithelium is exposed to a range of damaging agents and stressors (pathogens, environmental toxins, etc.), it requires robust mechanisms to repair damaged tissue and maintain homeostasis. Recent studies have shown that the adult murine intestine reactivates developmental gene expression programs following various forms of damage (helminth infection, dextran sodium sulfate, irradiation); however, repair mechanisms used by the adult human intestine remain unclear.

Aims: This study aims to define how the human intestine responds to diverse injuries, interrogate whether developmental gene expression programs are activated in response to damage/cell stress, and to identify the mechanisms that facilitate the injury response.

Method(s): Leveraging bulk RNA-seq data from patient-derived human intestinal organoids (epithelium only, termed 'enteroids') and previously published single cell RNA-seq data from primary tissue, I identify genes enriched in the developing human intestine relative to adult. ENRICHR and the Broad Institute Gene Set Enrichment Analysis (GSEA) tools were utilized to perform gene enrichment analyses. To define genes activated in response to cell injury, adult human enteroids were treated with 8 different conditions to induce various degrees of cell stress and damage. Bulk RNA-seq was used to define injury-specific gene programs and evaluate whether human fetal-enriched genes are activated in response to cell stress and injury. Additional bulk RNA-seq was performed to evaluate how activation of specific signaling pathways known to play a role in regeneration (TP53, YAP, TGFβ, PGE2) regulate human fetal-enriched genes.

Results: Analysis comparing adult and fetal in vivo and in vitro data revealed a core set of 140 fetal-enriched genes and145 adult-enriched epithelial genes. GSEA revealed that published fetal genes established from mouse enteroids exhibited a negative enrichment in our human fetal dataset. Follow-up studies treating adult human enteroids with 8 different stressors revealed that a handful of human fetal-enriched genes were elevated in response to specific injuries; however, there is an overall negative enrichment of human fetal genes in 6/8 treatment conditions. Furthermore, I found that 7/8 stressor conditions exhibited a strong positive enrichment for genes activated by TP53. Butyrate was the only exception and instead activated genes downstream of YAP and TGFβ signaling. Additional analyses revealed that stimulation of adult enteroids with TGFβ and YAP signaling in adult human enteroids promotes a more fetal gene signature whereas TP53 activation activates an adult gene signature.

Conclusions: My data supports that most of the cell stressors tested do not promote robust activation of human fetal-enriched genes in adult enteroids at the utilized timepoints and concentrations. Furthermore, I find that TP53 plays a central role in the intestine's response to injury and that TP53 activation promotes a more adult-like gene signature. Conversely, butyrate treatment promotes the activation of fetal-enriched genes, as well as genes downstream of YAP and TGFβ. Overall, my findings provide novel insight into the injury response programs utilized across a range of cell injury and stress stimuli in the human intestinal epithelium.

O. A novel model for inducing damage in human intestinal organoids in vivo using imaging to guide directed irradiation

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Abstract:
Villi are the finger-like projections in the small intestine that increase the absorptive surface area. During fetal development, the intestine begins as a flat sheet of endoderm with associated mesoderm that will fold and fuse to form a tube of epithelium surrounded by a tube of mesoderm. At embryonic day 14.5 in the mouse and 6-7 weeks in the human, aggregations of mesenchymal cells form adjacent to the epithelium and act as signaling centers to coordinate the emergence and outgrowth of villi from the flat epithelium. Human intestinal organoids (HIOs) are rapidly advancing as a model system for human intestinal development and disease, however, one limitation we are working to overcome is HIOs failure to emerge villi in vitro. When placed under the kidney capsule of a host mouse, these transplanted HIOs (tHIOs) form vasculature that connects to the host vasculature and they emerge villi with mature epithelial cell types. To study villus maintenance and repair, we needed a way to damage our tHIOs without inducing life-threatening illness in the host mice.

The Small Animal Radiation Research Platform uses bioluminescent imaging combined with Computed Tomography (CT) imaging to guide delivery of radiation specifically to the tHIO without damaging host bone marrow or other organs. We generated a plasmid to express luciferase-P2A-mCherry which was introduced into pluripotent stem cells along with a second plasmid to express piggy-Bac transposase to induce high-level incorporation of luciferase-P2A-mCherry into the genomic DNA of the stem cells. Stable transfectants were selected by puromycin resistance and then highly expressing cells were isolated by flow cytometry for mCherry. We then generated HIOs from the luciferase-P2A-mCherry expressing iPSCs and transplanted them into host mice. Using an IVIS bioluminescent imaging system, we were able to inject the host mice with luciferin and visualize the growth of our tHIOs based on bioluminescence. Once the tHIOs developed villi, we again used luciferase imaging of our tHIO overlaid with a CT image to precisely target irradiation to our tHIOs. Irradiated and control tHIOs were collected at 2, 6, 24, 48 and 72 hours post-irradiation, and damage and recovery were assessed by immunostaining and quantitative PCR for DNA damage, cell death, proliferation, stem cell, and cell type-specific markers. We found maximal damage occurred between 2-3 days post-irradiation and then recovery of proliferation and stem cell markers was observed. Our host mice never showed weight loss, abnormal stool or any other signs of illness. Using our luciferase expressing tHIOs, we are able to deliver directed irradiation to induce DNA damage specifically in our tHIOs and study the recovery process in vivo.

P. 3D-Printed Nested Transwell System for Immune Cell Infiltration into Lung Organoids

Lara Herlah (McGill University), Luc Mongeau (McGill University)

Abstract:
Respiratory infections, particularly those of the lower respiratory system, are among the most common infection-related causes of death worldwide. Even seasonal infections and milder cases cause a significant burden on the healthcare system. The COVID-19 pandemic showed that effective and accessible treatments of respiratory diseases take too long to develop; one of the reasons being a lack of an accurate model of the lungs. Animal models are often not representative of humans and 2D cell monolayers lack the complexity and heterogeneity intrinsic to the human lung. The development of accurate, heterogenous and infectable models of the human lungs is therefore crucial for the development of effective treatments and pharmaceuticals. Most organoid models, including lung organoids, lack immune cells; a key component when studying disease. Monocytes and macrophages are the most common immune cells found in the lung and play a significant role in both homeostasis and in pathological conditions. While macrophages are primarily recruited to help defend host tissue from infectious agents and aid in healing, in certain cases, pro-inflammatory macrophages can contribute to lung damage. Including these cells is therefore essential to accurately mimic infection conditions in the lung and develop treatments that are effective, efficient and safe. This study employs an infectable self-assembled heterogenous lung organoid that shows markers of both the proximal and distal lung regions and can be derived from either immortalised or primary cells. We aimed at establishing a lung organoid and monocyte co-culture system to investigate the role of monocyte migration and immune system activation in infected and non-infected organoids. The infiltration of monocytes into the lung organoid was performed using a nested transwell, i.e. a double transwell system where the organoid sits in one transwell and THP-1 monocytes are added to a second, separate transwell. Upon injury or infection, the organoid releases chemokines and pro-inflammatory cytokines that attract the THP-1 cells toward the organoid. THP-1 cells are able to extravasate through the transwell membrane, mimicking vascular extravasation of immune cells, making the model more representative of the human lung than direct co-cultures of monocytes and organoids. The study involved the design and validation of a 3D-printed nested transwell system through cell compatibility and extravasation assays. This allowed for an indirect co-culture of THP-1 cells and the lung organoid, which was used to evaluate chemokine-induced immune cell migration. Finally, the organoid and THP-1 cell co-culture was infected with the H1N1 influenza virus to compare the response of organoids to the infection with and without THP-1 cells. This approach establishes a framework for immune-integrated and infection-ready lung organoids that better recapitulate the human lung.

Q. Fc3TSR induces cytotoxicity of epithelial ovarian cancer patient-derived organoids

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Abstract:
Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy, challenged with advanced-stage diagnosis and low five-year survival rates. Treatment strategies for ovarian cancer have remained largely unchanged for decades and standard of care therapies suffer from limited efficacy and are prone to resistance. Our lab has developed an anti-angiogenic compound, Fc3TSR, which is a peptide fragment of the thrombospondin-1 protein. Through interactions with CD36, Fc3TSR exerts potent anti-angiogenic effects in vivo, leading to remodeling of the tumour microenvironment characterized by vascular normalization, reduced tumour hypoxia, and regression of advanced-stage disease in murine models of EOC. We have demonstrated that Fc3TSR also has pro-apoptotic functions against human and murine EOC cell lines. However, patient-derived organoids (PDOs) more closely recapitulate the complex three-dimensional structure, cellular heterogeneity, and genetic landscape of EOC tumours, and offer the unique opportunity to investigate therapeutic efficacy in patient-derived specimens. We assessed the cytotoxicity of Fc3TSR against high-grade serous carcinoma (HGSC) or ovarian clear cell carcinoma (OCCC) PDOs. PDOs were seeded in triplicate in a Matrigel dome at $3x10^4$ cells per well for the HGSC line or $2x10^4$ cells per well for the OCCC line. Cells were treated at 0nM, 250nM, 500nM, 750nM, or 1000nM. Following 48-hours of treatment, metabolic activity was assessed as an index of cytotoxicity by Alamar blue staining and absorbance was read with a microplate reader at 570nm and 600nm. Fc3TSR demonstrated dose-dependent cytotoxicity against the OCCC line (IC_{50}=621.2nM) and to a lesser extent in the HGSC line (IC_{50}>1000nM). While both PDOs expressed CD36, the OCCC line demonstrated higher levels of CD36 determined by immunoblotting. Here, we demonstrate that Fc3TSR induces cell death in OCCC and HGSC histotypes - an effect that may be dependent upon the expression of CD36. In follow-up to these findings, we will continue to test the efficacy of Fc3TSR against other PDO specimens to evaluate its broader therapeutic potential and assess molecular mechanisms of cell death to further elucidate histotype and inter-patient variability in the responsiveness to Fc3TSR.

R. Modeling the Impact of Maternal Diabetes on Planar Cell Polarity and Cardiogenesis Using Mouse Gastruloids

Loredana Cirillo, Qingming Ding, Xiangru Lu, Dean Betts, Qingping Feng

Abstract:
Gastruloids, 3D aggregates formed from self-organizing mouse embryonic stem cells (mESCs), serve as an in vitro model for early cardiogenesis offering precise spatial and temporal organization. When cultured with combination of growth factors including basic fibroblast growth factor (bFGF), ascorbic acid (AA), and vascular endothelial growth factor 165 (VEGF), gastruloids undergo cardiac differentiation. In this study, we use gastruloids as a model for embryonic heart development under normal and experimental conditions, with a focus on the impact of diabetes mellitus (DM).

Early heart development occurs shortly after gastrulation, when cardiac progenitors are specified and localization at the anterior of the embryo. This leads to the formation of a crescent-shaped domain – the first recognizable cardiac structure. Cardiogenesis progresses through coordinated interactions of the first and second heart field progenitors forming the cardiac crescent and subsequently rearranging into the linear heart tube – the beating structure. When mESCs are exposed to temporally regulated Wnt/β-catenin and BMP signalling, gastruloids derived from mESCs express cardiac progenitor genes including Nkx2.5, Mesp1, GATA4 and Tbx5. Additionally, mESC-derived gastruloids exhibit spontaneous beating and systolic calcium waves, physiologically mimicking early cardiac development.

Type 2 DM is a growing concern worldwide affecting 11.1% of adults and projected to rise to 13% by 2050. Furthermore, Pregestational Diabetes Mellitus (PDM) has been determined as a risk factor for multiple birth defects with congenital heart defects (CHDs) being the most likely at 1 in 5 births being affected. It was previously found that planar cell polarity (PCP) signalling, also known as the nonconical Wnt pathway, controls the spatial differences in cells through Rac1 modulation, and has shown to be critical for cell organization in the mesoderm with disruptions ultimately resulting in CHDs. Therefore, we hypothesized that a PDM-like environment impairs early heart morphogenesis by disrupting PCP signalling and causing abnormal cardiac progenitor organization and function. Through utilization of mESC-gastruloids exposed to increased glucose and insulin, simulating a diabetic environment, PDM and its effects can be investigated. This study aims to compare morphological, functional, and molecular features of gastruloids cultured in normal and high glucose conditions; to determine transcriptional changes caused by PDM-like conditions; and to investigate the dysregulation of PCP signalling in response to PDM-like conditions which link to CHDs. It is expected that under PDM-like conditions, gastruloids will display a loss of cell polarity, reduced beating frequency, and diminished contractile amplitude. PCP signalling and the related transcriptional network including mRNAs and miRNAs will be dysregulated. The study will provide mechanistic insights into embryogenesis and CHD pathogenesis during PDM.

S. STAT3 Driven Regulation of Lower Airway Epithelial Cells in the Developing Lung

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Abstract:
Recent progress in single cell genomic technologies has begun to uncover the heterogeneity of cell types and cell states within developing and adult human tissues at homeostasis and in diseased states. We have identified a unique SCGB3A2+SFTPB+CFTR+ progenitor secretory cell population, termed lower airway progenitor (LAP) cells in the developing lung using an 8 – 21 weeks post conception single-cell RNA sequencing (scRNA-seq) dataset. LAP cells are most abundant in the non-cartilaginous small airways and lineage tracing has shown LAP cells can give rise to pulmonary neuroendocrine cells (PNECs) and a subset of multiciliated cells more abundant in the distal airways. Despite recent advances in our understanding of LAP cell differentiation capacity, how LAP cells are regulated and the specific drivers behind the differentiation process remains unclear. Here, using an available multiomic (scRNA-seq + scATAC-seq) dataset from isolated distal regions of human fetal lungs enriched for LAP cells, the single-cell multiomic inference of enhancer and gene regulatory networks (SCENIC+) analysis pipeline was used to identify LAP cell-enriched transcription factors and predicted gene targets. Among prospective identified TFs, STAT3 was a top candidate based on its expression in vivo and functional experiments in organoids using pharmacologic inhibitors to test its role during differentiation. Additionally, we leveraged a spatial transcriptomics dataset to identify mesenchymal cells adjacent to pSTAT3+ LAP cells. By integrating these spatial data with multiome data, we employed CellChat to infer putative ligand-receptor interactions mediating communication between the neighboring mesenchyme and LAP cells. This analysis revealed candidate signaling pathways that may modulate LAP cell differentiation and self-renewal, providing new insights into the cellular crosstalk governing LAP cell fate decisions.

T. A novel method to measure Ca^{2+} entry in patient-derived pancreatic cancer organoids

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Abstract:
Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the 3rd leading cause of cancer-related death in Canada with a five-year survival rate of ~12%. One reason for this prognosis is the poor response tumours show towards current therapies. Novel targets suggested for treatment include pathways regulating intracellular stores of calcium (Ca^{2+}), an essential second messenger required for integrating signals from the extracellular environment into the regulation of signaling pathways and transcription factors. In cancer, elevated Ca^{2+} influx through ORAI1 channels at the plasma membrane via the store-operated Ca^{2+} entry (SOCE) pathway drives cell migration, proliferation and chemoresistance. While SOCE has emerged as a promising therapeutic target, this pathway has been primarily studied in PDAC monolayer cell lines, which does not properly capture the 3D nature of the tumour, the microenvironment (TME) and patient heterogeneity. Currently, no method for examining Ca^{2+} responses in PDAC patient-derived organoids (PDOs) has been documented. We developed methodology to assess the SOCE response in PDOs to address the hypothesis that Ca^{2+} responses will be heterogenous across different PDOs and influenced by cells within the TME.

Methods: Endoscopic ultrasound-guided fine-needle biopsies were obtained from patients undergoing PDAC diagnosis. The tissue was processed to isolate cancer associated fibroblasts (CAFs) or to establish 3D organoids grown in MatrigelTM. PDOs from different PDAC subtypes were selected for Ca^{2+} measurement. MatrigelTM was dissolved but organoids retained the 3D structure. These organoids were plated on glass-bottom dishes coated with poly-L-lysine, incubated with Fura-2 Ca^{2+} dye, and resting cytosolic Ca^{2+}, ER Ca^{2+} and SOCE were measured. The expression of SOCE regulators was examined with and without CAFs via immunofluorescent (IF) microscopy.

Results: We successfully developed methodology to measure cytosolic Ca^{2+} using Fura-2 in different PDO samples that showed differences between patients. Different areas within each PDO had similar Ca^{2+} responses, suggesting homogeneous Ca^{2+} signaling within a PDO. Interestingly, organoids with a simple epithelial layer showed increased SOCE response compared to PDOs showing more stratified epithelium, suggesting different PDAC phenotypes exhibit unique SOCE patterns. Conversely, resting cytosolic Ca^{2+} and ER Ca^{2+} did not correlate to cancer subtype. IF staining of CAFs and cancer cells in culture together resulted in increased staining in cancer cells compared to CAFs.

Conclusions: Our results reveal a new approach to assessing Ca^{2+} in PDAC PDOs and identified SOCE to be elevated in specific PDO phenotypes. These results are crucial to translating findings on SOCE inhibitors into clinical outcomes for patients.

U. Integrative drug screening and in vivo validation using patient-derived organoid and organoid-derived xenograft models of esophageal adenocarcinoma

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Abstract:

Background:

Esophageal adenocarcinoma (EAC) is an aggressive malignancy with rising incidence and poor survival rates. Although genomic profiling has identified recurrent driver alterations, effective targeted therapies remain limited. Our lab has previously demonstrated the generation of EAC patient-derived organoids (PDOs) from endoscopic biopsies, which recapitulate tumor histology, molecular features, and drug sensitivity (Derouet et al., 2020). This study aimed to establish and characterize a large biobank of EAC PDO and organoid-derived xenograft (ODX) models, assess their fidelity to patient tumors, and evaluate their potential as predictive tools for drug sensitivity and resistance.

Methods:

EAC tissue from >30 surgically resected and biopsy were collected from patients and processed to dissociate to single cells. Cells were embedded into Matrigel to generated PDOs and propagated under optimized 3D culture conditions. A panel of molecularly targeted agents, including inhibitors of EGFR, MAPK, PI3K, CDK4/6, ROS1, BRAF, PARP, and mTOR signaling pathways, was screened across these models to interrogate pathway-specific vulnerabilities. For these assays, cells were plated onto 384 well plates coated with Matrigel three days prior to treatment. Cell viability was measured four days post-treatment by CellTiter-Glo 3Dassay. For three drugs, 20-point dose-response assays were performed in five PDOs to validate reproducibility and sensitivity. To assess pathway inhibition, pEGFR and pHER2 or pERK1/2 was examined in afatinib- or trametinib-treated PDOs, respectively, by Western blot. To evaluate translational relevance, ODX models were established from 15 PDOs, achieving successful engraftment in 11. Whole-genome and whole-exome sequencing was performed to demonstrate concordance in gene mutation and copy number between matched tumors, PDOs, and ODXs. Histopathological analyses are underway to compare cellular morphology, differentiation, and biomarker expression patterns between patient tumors, PDOs, andODXs. Two ODX models were treated in vivo with trametinib and afatinib to compare with PDO drug screening results.

Results:

Drug screening across the PDO cohort revealed substantial intertumoral heterogeneity. Validation assays confirmed overall consistency of PDO drug responses. On-going Western blot analysis confirmed trametinib-induced inhibition of pERK1/2 in a trametinib-responsive PDO. In vivo drug responses were mostly consistent with in vitro findings: ODX model 1 was partially responsive to afatinib, yet strongly growth inhibited by trametinib, consistent with response of PDO; ODX model 2 exhibited marked tumor growth inhibition following afatinib, but resistance to trametinib. The corresponding PDO, however, exhibited growth inhibition to both drugs. These findings support the use of organoid-based drug testing to reliably predict in vivo treatment responses.

Conclusions:

This study will establish an integrated EAC organoid–xenograft platform that maintains patient tumor biology at genomic, histologic, and functional levels. The strong correlation between PDO drug responses and ODX outcomes supports the use of organoids as robust preclinical tools for predicting therapeutic efficacy. Ongoing profiling will elucidate mechanisms of drug sensitivity and resistance. These findings support the integration of organoid-based research into translational research and drug development for EAC.

V. Generating Vascularized Skeletal Muscle Organoids from Patient-Derived PBMCs to Model Duchenne Muscular Dystrophy (DMD)

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Abstract:
Duchenne muscular dystrophy (DMD) is the most common and severe form of muscular dystrophy, affecting ~30,000 boys in North America. This X-linked recessive disorder is caused by mutations in the DMD gene, which encodes dystrophin, a large cytoskeletal protein essential for preserving muscle fiber integrity. Without dystrophin, skeletal and cardiac muscle fibers are highly susceptible to contraction-induced injury, leading to necrosis, incomplete repair, and replacement of muscle with fibrotic and fatty tissue. Patients experience progressive muscle weakness, loss of ambulation, respiratory failure, and cardiomyopathy. Despite research advances, DMD remains a fatal condition, with most patients not surviving beyond their third decade of life.

Gene replacement therapies such as microdystrophin (μDys) offer partial benefit but do not fully address disease complexity. Impaired vascularization further exacerbates progression: loss of dystrophin disrupts nitric oxide signaling, reducing blood flow and causing ischemia, while chronic inflammation and excessive collagen deposition drive irreversible fibrosis. Angiopoietin-1 (Ang-1), a protein critical for blood vessel formation and maturation, is depleted in dystrophic muscle. Restoring Ang-1 signaling alongside μDys therapy may synergistically stabilize muscle fibers and improve vascular support.

To test this therapeutic concept in a human-relevant system, we will generate vascularized skeletal muscle organoids from patient-specific induced pluripotent stem cells (iPSCs). This approach avoids the ethical limitations of muscle biopsies, overcomes the translational shortcomings of the dystrophin-deficient mdx mouse, and provides a customizable platform for therapy testing.

Objectives:
1. Generation of iPSCs: Peripheral blood mononuclear cells (PBMCs) will be isolated from two DMD patients and reprogrammed into iPSCs.
2. Skeletal muscle differentiation: iPSCs will be differentiated into myogenic progenitors and multinucleated myotubes. Healthy control skeletal muscle organoids will be derived from established iPSC lines.
3. Vascular organoid development: iPSCs will be differentiated in parallel into endothelial cells and pericytes to generate vascular organoids.
4. Integration: Vascular and muscle organoids will be co-cultured to generate vascularized skeletal muscle organoids that recapitulate human muscle structure and function.
5. Therapeutic testing: This platform will be used to evaluate Ang-1 supplementation combined with μDys, with endpoints including vascularization, fibrosis reduction, and dystrophin restoration.

Over seven million PBMCs were successfully isolated from two DMD patients, providing a robust starting population. Myogenic differentiation of established PBMC-derived iPSCs (SCTi003-A) produced three-dimensional colonies within nine days. Although proliferation plateaued thereafter, likely due to high seeding density, these results demonstrate feasibility. Additional iPSC lines have since been obtained for further practice and method optimization, laying a technical foundation for robust myogenic and vascular differentiation. Next steps include refining culture conditions, confirming pluripotency, and assessing lineage-specific differentiation.

Integrating vascular and myogenic components into patient-derived organoids overcomes the limitations of animal models and biopsies, providing a physiologically relevant, ethically responsible, and customizable platform for DMD research. It will advance our understanding of disease mechanisms, accelerate testing of personalized therapies, and transform research and treatment options.

W. Delivery of induced pluripotent stem cells in cell-instructive composite hydrogels for nucleus pulposus regeneration

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Abstract:
Chronic back pain is the leading cause of lifetime disability, caused by intervertebral disc (IVD) degeneration, specifically attributable to the degeneration of the nucleus pulposus (NP), the innermost gel-like IVD structure. Poor endogenous repair mechanisms of the IVD, along with limitations of current treatments focused primarily on alleviating symptoms, motivate our investigation of cell-based therapies targeting the NP to restore the structure and function of degenerated IVD. Human induced pluripotent stem cell (iPSC)-derived populations have potential as a promising clinically-translational cell source, due to their regenerative potential and feasibility of autologous sourcing. This study aims to develop in situ-forming mechanically-resilient hydrogels incorporating micronized decellularized nucleus pulposus (DNP) sourced from bovine IVD as a cell-instructive platform to guide human iPSC-derived notochordal-like (NC-like) or NP-like cell populations to regenerate the IVD. We hypothesize that the iPSC-derived NC-like cells will exhibit enhanced regeneration compared to iPSC-derived NP-like cells following hydrogel encapsulation and culture under conditions mimicking the native human NP. We further hypothesize that incorporation of DNP particles will augment regeneration. A hydrogel based of 4-arm [poly(ethylene glycol)-b-poly (trimethylene carbonate)-acrylate] (4a[(PEG-b-PTMC)-Ac] and thiolated chondroitin sulphate (CS-SH) will be used as the basis of our platform. We will incorporate varying DNP contents (0, 3, 5, 10%) to establish formulations matching the mechanical properties of human NP tissue that support the viability and phenotype of encapsulated iPSC-derived NC- and NP-like cells. Physiological loading associated with daily activities will be applied to composite hydrogels using a high-throughput bioreactor to identify the cell source (NC-like or NP-like) and platform (gel+DNP or gel alone) that enhances NP markers, matrix production, and biomechanical properties. Our preliminary studies have established a detergent-free decellularization protocol that preserves the biochemical composition of the ECM while removing cells, resulting in scaffolds rich in structural ECM components. We have also demonstrated that the compressive modulus of the hydrogel composites can be tuned to be within the range of that reported for human NP with varying gel and DNP concentrations. Ongoing experiments are focused on testing the differentiation of human iPSCs to NC- and NP-like cells and the effects of 3Dculture in composite hydrogels on cell phenotype and ECM production. Combining decellularized NP ECM with synthetic polymers has the potential to capitalize on the favourable properties of both to generate cell-instructive composites that satisfy all requirements for a clinically-relevant NP regenerative cell therapy platform.

X. Time-resolved genomic, transcriptomic, and microenvironmental landscape during HPV-induced carcinogenesis.

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Abstract:
Background: Human papillomavirus-positive head and neck squamous cell carcinoma (HPV+ HNSCC) is a growing health concern in Canada. A critical knowledge gap exists in understanding the initial molecular events of carcinogenesis due to the lack of a clinically evident premalignant lesion and suitable experimental models. The palatine tonsils are among the most common sites for HPV-related oropharyngeal cancers. Their lymphoepithelial structure, rich in immune cells and crypts, makes them particularly susceptible to persistent HPV infection. We propose to establish a novel, immune-competent tonsil-derived organoid platform to model the early stages of HPV-driven transformation.

Methods: Tonsil tissue will be obtained from consenting patients undergoing elective tonsillectomy. Organoids will be generated by co-culturing epithelial cells, transduced withHPV16 E6/E7 lentiviruses, with their native epithelial-depleted immune cell populations. We will employ a longitudinal study design, performing whole-genome sequencing (WGS) and single-cell RNA sequencing (scRNA-seq) at early, intermediate, and late timepoints post-transduction to map the evolution of genomic and transcriptomic changes.

Preliminary Data: We have successfully established a robust protocol for generating tonsil organoids from primary tissue, with cultures remaining viable for 4-6 weeks. This demonstrates the feasibility of our proposed long-term experiments.

Expected Outcomes: This study is expected to deliver a high-resolution, time-resolved map of the molecular and cellular events driving HPV-induced carcinogenesis. We anticipate identifying novel genomic and transcriptomic biomarkers for early disease detection and potential therapeutic targets. The integration of multi-omics data will provide unprecedented insight into tumor-immune dynamics during the initial phases of malignant transformation.

Conclusion: This research will establish a physiologically relevant platform for studying HPV+ HNSCC initiation. The findings will advance our fundamental understanding of HPV-driven cancer and lay the groundwork for developing innovative strategies for early detection, prevention, and personalized therapy.

NOTES:

Thank you on behalf of our entire team!

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