Oz Single Cell

2022 Annual Conference

Q1 Resort, Gold Coast

14 - 16 September 2022

Program and Abstract Book

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Oz Single Cell

Welcome!

Dear colleagues,

After two years of virtual meetings, we are very excited to welcome you to Oz Single Cell 2022 on the beautiful Gold Coast. This year's conference brings together the ever-growing Australian community to discuss the latest developments and applications pushing the boundaries of single cell science.

With our stellar line-up of international and national speakers, as well as over 90 posters showcasing a wide range of cutting-edge research from PIs and ECRs alike, we have assembled a topical and multi-disciplinary program where there is plenty of exciting science for everyone. Our packed social program also promises opportunities for extra laughs, a bit of competitive spirit from opinion leaders, and networking opportunities to foster collaboration and career friendships. Our sponsors bring with them not only valuable support, but the important updates and opportunities on their rapidly developing technologies that will help drive your own research further.

We believe that you find this conference as rewarding as it has been to put together. Returning to a face-to-face meeting format is especially meaningful for our Oz Single Cell community and we hope that the stunning vies of the Q1 tower will help inspire you to new scientific heights!

Please don't forget to share your experiences throughout the meeting with us using the hashtag #OzSingleCell22 or tweet us at @ozsinglecells!

Welcome to the Gold Coast; it'll be an exciting one, and definitely worth the wait!

The Oz Single Cell 2022 Organising Committee

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The Oz Single Cell Organising Committee would like to thank the above organisations for their support of the Oz Single Cell 2022 Annual Conference.

Acknowledgement of Country

We acknowledge the Traditional Owners and their custodianship of the land on which we live, work and gather. We pay our respects to their elders both past, present, and emerging, who continue cultural and spiritual connections to Country. We extend that respect to all Aboriginal and Torres Strait Islander Australians and recognise their valuable contributions to Australian and global society.

Code of Conduct

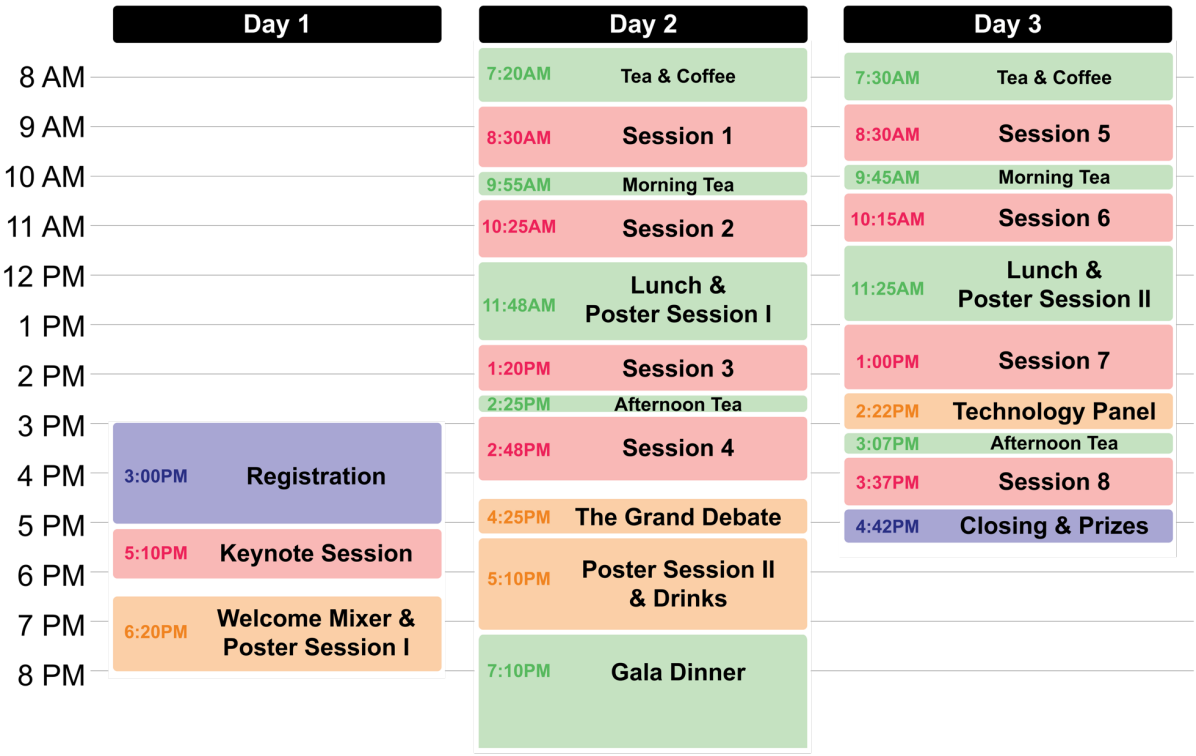
Oz Single Cell is all about community, collaboration, and innovative possibilities when we all come together. To make this conference a safe and welcoming space for all, we ask you to abide by our code of conduct. We respect the safety and privacy of all delegates, will treat each other with respect and without prejudice for gender, ethnicity, career stage, or academic discipline. Harassment and bullying will not be tolerated. We invite sharing of unpublished data and approaches and ask delegates to participate with honesty and openness to new ideas. We ask delegates to share their experiences on social media whilst respecting presenter wishes about sharing of data on these platforms.

COVID-19 Policy

While we meet, the team at SkyPoint and the Oz Single Cell organising committee encourage COVID Safe practices and strive to ensure a healthy and safe environment is maintained at all times. The team at SkyPoint have worked with government health authorities to develop robust procedures to maintain cleanliness and reduce the risk of spread. In keeping with government guidelines, we recommend delegates to practice good hygiene, carry a mask on them and wear it when unable to physically distance indoors, and refrain from attending the conference if feeling unwell. For more guidelines and information, please visit the Queensland Health website.

Oz Single Cell

Program at a glance



Day 1 Schedule

3:00 PM	Registration
5:00 PM	Opening
	Keynote Session *Chairs: Joseph Powell & Atefeh Taherian Fard*
5:10 PM	Peter Kharchenko Analysis of somatic copy number variations from single-cell transcriptomics
5:40 PM	Melissa Davis An analysis of Spatial Single Cell Transcriptomics in Non-small cell lung cancer *Sponsored by NanoString*
6:05 PM	Lightning Talks I *Chair: Alicia Oshlack*
6:20 PM	Welcome Mixer & Poster Session I *Sponsored by NanoString*

14th September

DAY 1

Day 2 Schedule

7:20 AM	Tea and Coffee
8:20 AM	Opening
	Theme 1: Systems Biology at the Single Cell Level *Chairs: Shalin Naik & Drew Neavin*
8:30 AM	Joakim Lunderberg Tracking early events in cancer by spatial omics
9:00 AM	Ankur Sharma Oncofetal ecosystem in liver cancer: spatial localisation and clinical implications
9:25 AM	Anna S.E. Cuomo Uncovering context-specific and dynamic genetic regulation of gene expression in PBMCs at single-cell resolution
9:40 AM	Carolyn de Graaf Comparison of haematopoietic cells across vertebrates using single cell transcriptomes
9:55 AM	Morning Tea
	Theme 2: Developmental biology, cell fate, and lineage tracing *Chairs: Arutha Kulasinghe & Jen Currenti*
10:25 AM	Delphine Merino Single cells: their mother/daughter relationships and interactions with their neighbours revealed by cellular tracking
10:50 AM	Geoffrey McDermott Accessing new sample types to expand your research: Single Cell Fixed RNA Profiling and Visium CytAssist *Sponsored by 10x Genomics*
11:08 AM	Nathan Palpant Unsupervised methods to determine cell-cell and gene-gene relationships
11:33 AM	Sara Tomei LoxCode barcoding resolves maintenance of the long-term haematopoietic stem cell pool through symmetric self-renewal
11:48 AM	Lunch & Poster Session I *Sponsored by Akoya/Geneworks*
	Theme 3: Single cell research at the population scale *Chairs: Belina Phipson & Walter Muskovic*
1:20 PM	Seyhan Yazar Single-cell eQTL mapping identifies cell type-specific genetic control of autoimmune disease
1:45 PM	Longqi Liu High-resolution panoramic spatial transcriptomics using DNA nanoball patterned array *Sponsored by BGI-Decode*
2:10 PM	Nadia Rajab Proliferation is a driver of quorum sensing in an in vitro model of tissue resident macrophages
2:25 PM	Afternoon Tea *Poster changeover for Poster Session II*
	Theme 4: Single cell for precision medicine *Chairs: Jovana Maksimovic, Xiao Tan*
2:48 PM	Anuja Sathe Understanding heterogeneous cellular responses to cancer immunotherapy
3:18 PM	Oliver Braubach Advancing spatial biology through true single-cell spatial multi-omics *Sponsored by Akoya/Geneworks*
3:30 PM	Jacky Li Benchmarking of in vitro human hematopoiesis from pluripotent stem cells
3:55 PM	Raymond Louie Multi-omic profiling of rogue lymphocytes in celiac disease
4:10 PM	Lightning Talks II *Chair: Alicia Oshlack*
4:25 PM	The Grand Debate *Team captains: Alicia Oshlack & Tom Ashhurst; Moderator: Shalin Naik* *Sponsored by In Vitro Technologies/ACDBio*
5:10 PM	Poster Session II *Sponsored by BGI-Decode Science*
7:10 PM	Gala Dinner *Sponsored by 10x Genomics & Illumina*

15th September

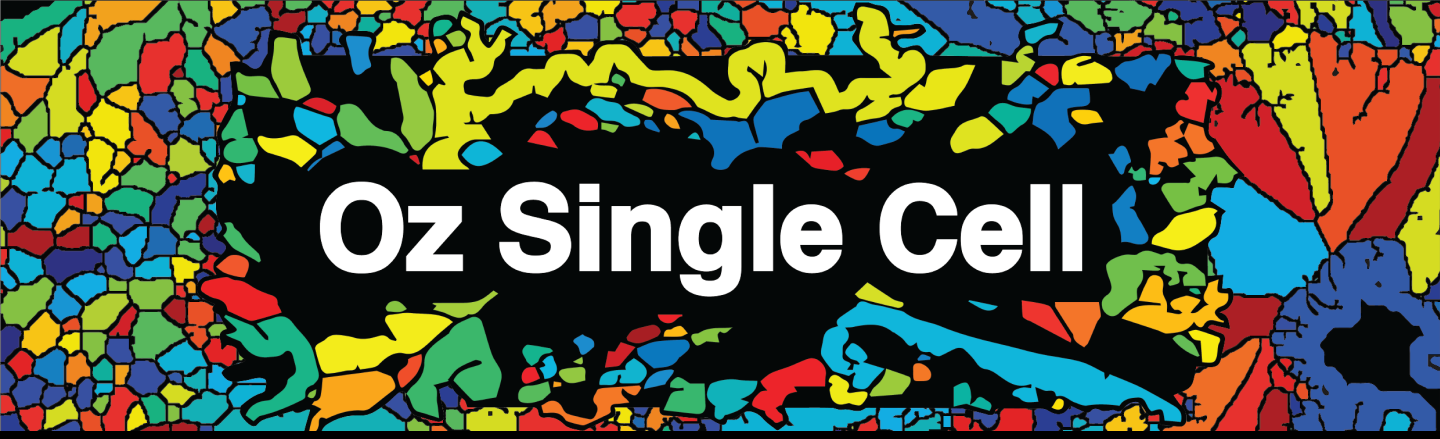
DAY 2

Day 3 Schedule

7:30 AM	Tea and Coffee
8:25 AM	Opening
	Theme 5: Building and making use of single cell atlases *Chairs: Pengyi Yang & Vera Sun*
8:30 AM	Mohammad Lotfollahi Multi-scale learning on single-cell atlases: from cells to patients
9:00 AM	Guiyan Ni Comparative spatial multiomics analyses of a cell atlas and interactome in skin cancer reveal cell types, gene markers, and cell-cell interactions underlying skin cancer diversity
9:15 AM	Daniel Roden A single-cell and spatially resolved atlas of human breast cancers
9:30 AM	Oleg Gusev Brain cell atlas of an anhydrobiotic insect: cell-type-specific mechanisms of complete desiccation tolerance and insights for new neuroprotectors for treatment of reperfusion syndrome.
9:45 AM	Morning Tea
	Theme 6: Single cell applications *Chairs: Heather Lee, Ahmed Mehdi*
10:15 AM	Hamish King Single cell genomics to map B cell maturation and etiology of autoimmune risk loci
10:40 AM	Alistair Forrest A cellular and spatial atlas of mammalian tongue *Sponsored by Vizgen/Integrated Bioscience*
10:55 AM	Justin Rubio The mutational landscape of single neurons and oligodendrocytes reveals evidence of inflammation-associated DNA damage in multiple sclerosis
11:10 AM	Sophie Shen An integrated cell barcoding and computational pipeline for scalable analysis of differentiation at single-cell resolution
11:25 AM	Lunch & Poster Session II
	Theme 7: Beyond single cell RNA sequencing *Chairs: Saskia Freytag & Rodrigo Carlessi*
1:00 PM	Catherine Wong New version of Glass-Oil-Air-Droplet (GOAD) nano chip device and some applications
1:30 PM	Scott Berry Multiplexed protein imaging from the subcellular to the cell-population scale
1:55 PM	Amrita Roy From Seq to Spatial Insight: Interrogate novel cell phenotypes and spatial RNA signatures at single-cell resolution with gold-standard RNAscope technology *Sponsored by In Vitro Technologies/ACDBio*
2:07 PM	Luyi Tian Spatial transcriptomic maps of whole mouse embryos
2:22 PM	Technology panel *Chair: Luciano Martelotto* *Sponsored by Vizgen/Integrated Sciences*
3:07 PM	Afternoon Tea *Posters to be taken down*
	Theme 8: Emerging research directions in single cell *Chairs: Tom Ashhurst & Rhea Pai*
3:37 PM	Jean Yang Atlas-scale data integration for single-cell meta analysis *Sponsored by Illumina*
4:02 PM	Irina Kuznetsova Benchmarking recently available alternative single cell/nuclei profiling kits
4:17 PM	Ryan Lister Molecular dynamics of human brain development
4:42 PM	Concluding Remarks and Prizes

16th September

DAY 3



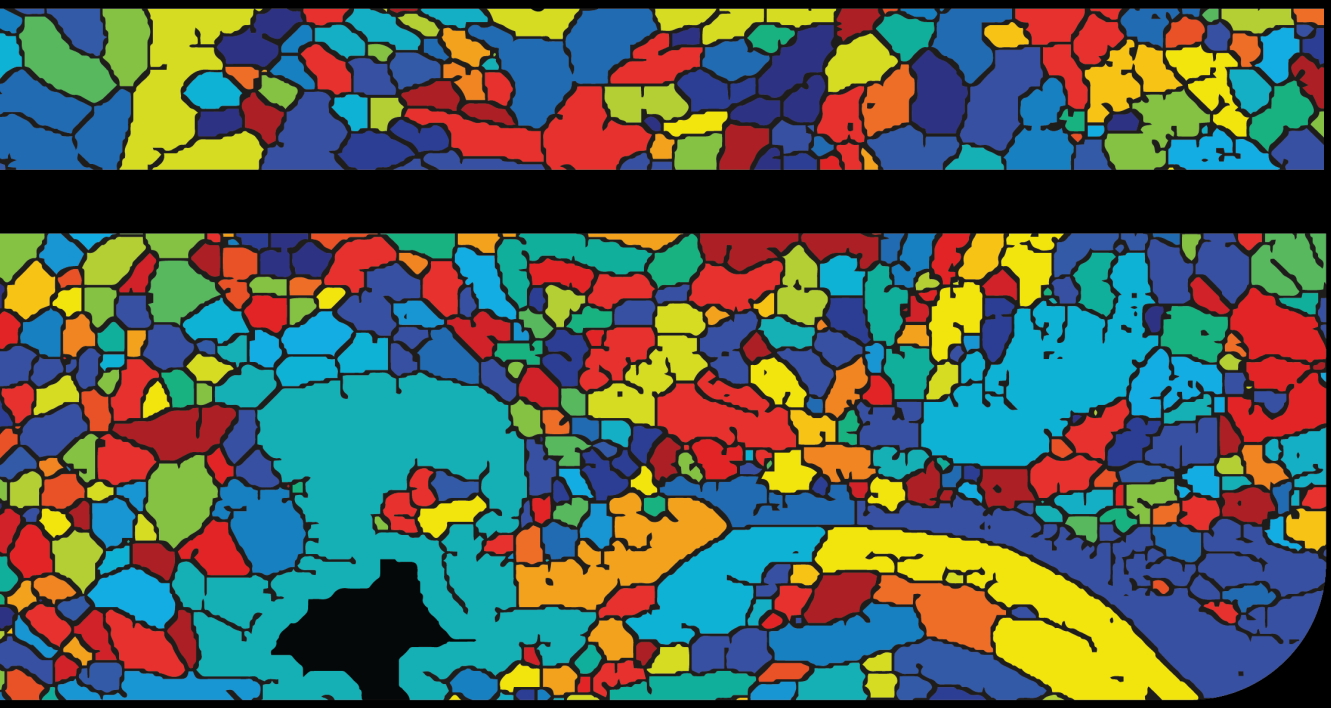
Poster Session I

	#	Presenter	Poster Title
Lightning Talks	1	Alanna Spiteri	Temporal tracking of microglial and monocyte single-cell transcriptomics in lethal flavivirus infection
	2	Duy Pham	Spatio-temporal landscape of cellular interaction and immune cell dynamics in traumatic brain injury
	3	Cindy Audiger	Single-cell RNA sequencing reveals how conventional dendritic cell heterogeneity is affected during systemic inflammation
	4	Cameron G. Williams	Defining lymphocyte differentiation in the spleen during malaria with spatial transcriptomics at near single-cell resolution.
	5	Chenhao Zhou	Use spatial transcriptomics to study tumor predictors in patients with early invasive melanoma
	6	Claire Wishart	Connecting transcriptomes across neuroinflammatory disease reveals conserved and unique microglial and monocyte response programs
	7	Dalia Mizikovsky	Analysis of population genetic and phenotypic diversity reveal a predictable organisation of gene programs in the cell
	8	Dane Vassiliadis	Deciphering the non-genetic determinants of malignant clonal dominance and therapeutic resistance
	9	Drew Neavin	Demuxafy: Improvement in droplet assignment by integrating multiple single-cell demultiplexing and doublet detection methods
	10	Dylan Sheerin	Convalescent individuals with previous severe COVID-19 have increased abundance of interferon-expressing neutrophils in circulation
	11	Hsiao-Chi Liao	A toolbox for analysing single-cell proteomics data from LEGENDScreen assay
	12	Enakshi Sinniah	A Genome-wide Epigenetic Repressive Signature Reveals Genetic Regulators of Cell Identity at Single Base Resolution
	13	Harry Mueller	Nuclei Segmentation Techniques for Improving STOmics Analyses
	14	Huiwen Zheng	Leveraging single-cell RNA-sequencing atlases to understand gene essentiality
	15	Lucas Tobar	Single-cell RNAseq of adult mouse brain and meningeal vasculature
	31	Agus Salim	RUV-III-NB: normalization of single cell RNA-seq data
	32	Alejandro Casar	Identifying gene expression programs associated with transcriptional plasticity in single cell RNA sequencing data
	33	Andy Tran	scREMOTE: Using multimodal single cell data to predict regulatory gene relationships and to build a computational cell reprogramming model
	34	Angli Xue	Pitfalls and opportunities for applying PEER factors in single-cell eQTL analyses
	35	AnneMarie Welch	Transcriptional Steps in Megakaryocyte Commitment and Maturation
	36	Ashley Soet	GeoSpatial-scRNA-seq reveals distinct spatial patterning of the tumor microenvironment in hepatocellular carcinoma
	37	Atma Ivancevic	Endogenous retroviruses mediate transcriptional rewiring in response to oncogenic signaling in colorectal cancer
	38	Beata Kiedik	Building a cellular atlas of localised luminal breast cancers
	39	Brad Balderson	Detection of significantly different populations of cells in single cell RNA-seq data
	40	Carissa Chen	Knowledge-based Single-cell Clustering and Annotation
	41	Cecilia Gomez Inclan	Elucidating the relationships between markers of cellular senescence in human esMSC undergoing replicative senescence
	42	Chunlei Liu	Matilda: Multi-task learning from single-cell multimodal omics
	43	Daniel Brown	A comparison of cell multiplexing reagents for scRNA-seq
	44	Dannel Yeo	Mutational Profiling of Single Pancreatic Cancer Circulating Tumour Cells
	45	Di Xiao	Time-resolved phosphoproteome and proteome analysis reveals kinase signaling on master transcription factors during myogenesis
	46	Dmitrii Shek	Multi-omic signatures of immune-checkpoint inhibitor therapy in patients with malignant pleural mesothelioma.
	47	Enikő Regényi	Ellipse representation for detecting changes in expression and splicing in colorectal cancer
	48	Esmaeel Azadian	Identifying universal molecular regulators of ‘clonal wave’ properties
	49	Eva Apostolov	Investigating the cellular and AR-activity landscape of treatment-naïve localised prostate cancer using single-cell RNA-seq
	50	Fumihiko Takeuchi	Single-nucleus ATAC-seq elucidates major modules of gene regulation in the development of non-alcoholic fatty liver disease
	51	George Howitt	#fail or #success? Pitfalls and pointers for HTO demultiplexing of scRNA-seq data
	52	Habib Sadeghirad	High-plex spatial proteomic profiling of immunotherapy response groups in head and neck cancer identifies tissue signatures associated with therapy response
	53	Hani Kim	Comprehensive Characterisation of Fetal and Mature Retinal Cell Identity to Assess the Fidelity of Retinal Organoids
	54	Holly Pearson	UTS Single Cell Technology Facility: Providing personalised solutions for single cell multiomics
	55	Hue Mai La	Defining the immune milieu of pancreatic adenocarcinoma using single-nuclei RNA sequencing
	56	HuiQi Hong	Using Single-cell Gene Expression to Develop Treatment Response Predictions in Multiple Myeloma
	57	Jacqueline Tearle	Mapping Rare Inflammatory Bowel Disease Pathologies at Single Cell Resolution
	58	Jarny Choi	No time to retire: bulk buying in the age of single cell
	59	Jeffrey Pullin	A comparison of marker gene selection methods for single-cell RNA sequencing data
	60	Jessica von Pein	Dual single-cell RNA sequencing to characterize molecular mechanisms of the macrophage zinc toxicity antimicrobial response against intracellular Escherichia coli

Poster Session II

	#	Presenter	Poster Title
Lightning Talks	16	Igor Adameyko	Solving the three of transcriptional events and decision-making for neural crest lineage
	17	James Monkman	Multi-omic and spatial dissection of immunotherapy response groups in non-small cell lung cancer (NSCLC)
	18	Jennifer Currenti	STOmics identifies response associated cellular neighbourhoods in Hepatocellular Carcinoma
	19	Jovana Maksimovic	Single-cell RNAseq of amniotic fluid: a sneak peak into fetal development and disease
	20	Laura Grice	Spatial omics reveals differential fibrotic responses to spinal cord injury that are lesion-level-dependent
	21	Minh Tran	Spatially-resolved cell-cell interaction analysis of cancer tissue morphology
	22	Neda Rahmani Mehdiabadi	Defining the fetal gene program at single cell resolution in pediatric dilated cardiomyopathy.
	23	Rhea Pai	A single cell and spatial atlas of Hepatocellular Carcinoma across aetiologies
	24	Taopeng Wang	An experimental perspective of the Digital Spatial Profiling (DSP) and Visium technologies
	25	Walter Muskovic	Dissecting the drivers of intra-tumoural heterogeneity in high grade brain tumours with single cell RNA-seq
	26	Yue Cao	scFeatures: Multi-view representations of single-cell and spatial data for disease outcome prediction
	27	Yuliangzi Sun	Inferring cell diversity in single cell data using consortium-scale epigenetic data as a computational anchor for cell identity
	28	Yupei You	Identification of cell barcodes from long-read single cell RNAseq with BLAZE
	29	Zhixuan Wu	Identifying genetic features to predict and control cell differentiation lineages from pluripotency
	30	Yue You	Modeling group heteroscedasticity for pseudo-bulk samples in single-cell RNA-seq data
	61	Jin Ng	Interrogating immune cell heterogeneity of human small cell lung cancer
	62	Jinming Cheng	Time course analysis of pseudo-bulk samples from single cell RNA sequencing data
	63	Kristen Feher	Comparing single cell datasets using DensityMorph
	64	Lachlan Gray	Investigating the influence of X chromosome inactivation on the female bias in autoimmune disease
	65	Laura Rodriguez de la Fuente	Myeloid-derived suppressor cells regulate metastatic dissemination in triple-negative breast cancer
	66	Lipin Loo	Profiling Cellular and Molecular Mechanisms of Chronic Pain with Integrated Transcriptomics
	67	Louise Baldwin	Multi-omic characterization of the response to immunotherapy in murine breast cancer models
	68	Shabarni Gupta	TrajectoryNet reveals temporal transcriptional relationships driving the mesenchymal-to-epithelial transition in tumour formation
	70	Masih Sherafatian	Deconstructing epithelial ovarian cancers using single cell gene expression data
	71	Matthew E Jones	Comparison of 10x Genomics 3'v3.1 and ParseBio Evercode technologies on myoblast nuclei
	72	Natalie Charitakis	Disparities in spatially variable gene calling highlight the need for benchmarking methods
	73	Niko Thio	Streamlining end-to-end CyTOF data analysis
	74	Nona Farbehi	Population scale stem cell biology with induced pluripotent stem cells during cardiac differentiation
	75	Noorul Amin	Flexiplex: A Flexible Demultiplexer and Search Tool for Single Cell Data
	76	Onkar Mulay	STimage: deep learning inferences of cancer gene and cell markers using histological tissue images and spatial transcriptomics data
	77	Osvaldo Contreras	Temporal transcriptomic dynamics of the ATX-LPAR-PLPP axis during skeletal muscle regeneration at single cell resolution
	79	Raisa Hasan	Investigation of metastatic-castration resistant prostate cancer heterogeneity using single-cell technologies
	80	Ruqian Lyu	Personalised haplotype assembly and comparative crossover map analysis using single-gamete sequencing data
	81	Sagrika Chugh	scATACPop: Simulating population scale single-cell ATAC seq data
	82	Saskia Freytag	Uncovering subtype specific intra-tumoural heterogeneity in brain cancer
	83	Shokouh Shahrokhi Sabzevar	Mapping cell-type specific transcriptomic signatures and defining differences between genetic subtypes in brain tissues of individuals with Prader-Willi syndrome.
	84	Shuyi Wu	Identifying gene fusions in single-cell RNA sequencing of cancers.
	85	Soon Wei Wong	Single-nucleus Transcriptome Profiling of Kidneys Undergoing Compensatory Growth
	86	Stephen Zhang	Inferring cell-specific causal regulatory networks from drift and diffusion
	87	Tom Weber	Synthetic single-cell tools to decipher clonal fate programs
	88	Tuan Vo	Generation of spatial transcriptomics data from skin cancer and melanoma fixed tissues to add molecular signatures to pathological annotation
	89	Xiangnan Xu	scMoE: A regularized mixture of experts model to identify heterogeneous relationship between cell type proportion and host health in COVID-19 patients.
	90	Xiao Tan	A novel computational approach for integrative analysis of spatial multi-omics data highlights immune responses to SARS-CoV-2 infection in lung tissue
	91	Yang Yang	Improving UMAP by preserving feature information enhances single cell data analyses

Day 1 Talks



Invited Speakers

Peter Kharchenko

Department of Biomedical Informatics, Harvard Medical School

Peter received a PhD in Biophysics at Harvard University, studying gene regulation and metabolic networks under the advisement of George Church. He then completed a four-year postdoctoral fellowship in computational biology and genomics in the laboratory of Peter Park.

Analysis of somatic copy number variations from single-cell transcriptomes

Melissa Davis

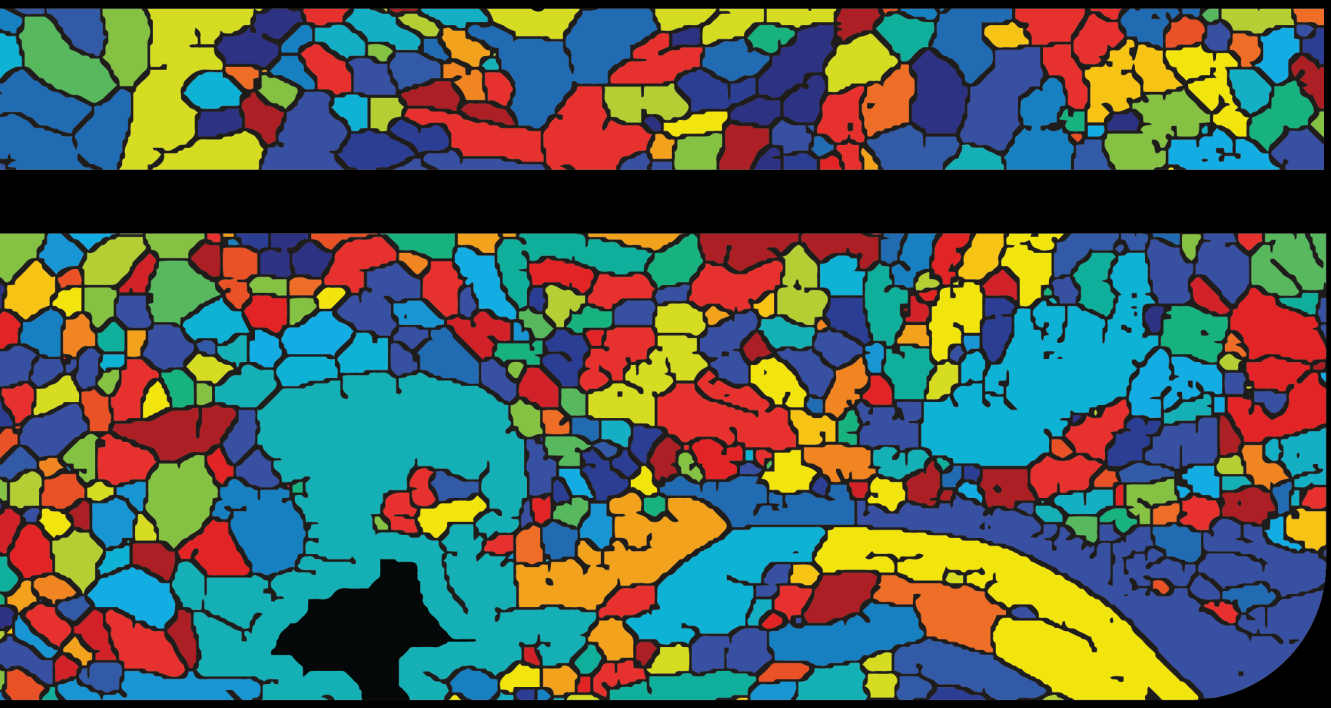
Walter and Eliza Hall Institute of Medical Research

Professor Melissa Davis is a computational biologist and group leader at the Walter and Eliza Hall Institute, where she leads a highly multidisciplinary research team focused on computational research in cancer progression and plasticity. She is also Joint Division Head of the WEHI Bioinformatics Division, a research division comprising >60 bioinformaticians, biostatisticians and computational scientists as well as research higher degree students and visiting scientists across seven research labs and a core Bioinformatics Facility. She has recently been appointed as Professor in the Faculty of Medicine at the University of Adelaide, and in 2023 will take up an appointment as Program Leader in Computational Systems Oncology in the newly established South Australian ImmunoGenomics Cancer Institute (SAiGENCI), where she will be a part of the senior leadership team recruited to establish Australia's first new medical research institute in over a decade. Melissa is recognised nationally and internationally for her work in computational biology and network analysis, knowledge engineering, cancer plasticity and methods development for spatial transcriptomics.

An analysis of Spatial Single Cell Transcriptomics in Non-small cell lung cancer

Spatial transcriptomics provides an unprecedented window into the structural organisation of cells within tumours, and new technologies now enable the analysis of molecules at single cell resolution. Using data from the Nanostring CosMx SMI Platform we have analysed spatial transcriptomes for five NSCLC samples. I will discuss the unique characteristics of this new type of single cell data, which are of a fundamentally different type to traditional sequencing-based methods and present unique analytical challenges. These technologies will require a conceptual shift from traditional single cell technologies.

Day 2 Talks



Invited Speakers

Joakim Lundeberg

Science for Life Laboratory, Division of Gene Technology, KTH Royal Institute of Technology, Stockholm, Sweden

Joakim Lundeberg, Ph.D., Professor in Molecular Biotechnology, was a co-founder of Science for Life Laboratory Sweden in 2010, a national and multi-university effort in large-scale life sciences providing access to infrastructures such as genomics, and proteomics, imaging, metabolomics, and drug development. Dr. Lundeberg has, during the most recent years, focused on spatial transcriptomics that enables a detailed description of gene expression patterns in tissue sections. Dr. Lundeberg has publications demonstrating the development of the technology but also examples of the impact of spatial analysis in biology. The research focuses on expanding the spatial modalities and developing new software tools and applications in human cell atlas, neurology, and cancer. JL is currently a visiting scientist in Alex Swarbricks group at Garvan Institute, Sydney.

Tracking early events in cancer by spatial omics

Ankur Sharma

Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, 6 Verdun Street, Nedlands, Perth, Western Australia 6009, Australia

Dr Ankur Sharma is a Lab Head of the Onco-Fetal Ecosystem Laboratory at Harry Perkins Institute of Medical Research and Curtin University. Ankur is also the Genomics Lead of Liver Cancer Collaborative.

Ankur's research is directed towards exploring the similarities between fetal and tumor microenvironment. He is specifically interested in understanding the Onco-Fetal reprogramming of the tumor ecosystem. Ankur is one of the earlier adopters of single-cell genomics to understand the evolution and ecosystem of tumors. In 2019, he was awarded the NMRC Young Investigator fellowship and in 2020, was appointed as a Research Scientist at Spatial and Single Cell Systems Domain at GIS, ASTAR. Ankur's work led to the discovery of oncofetal reprogramming of tumor ecosystem in liver cancer (Sharma et al., Cell 2020; Sharma et al., Nature Reviews Cancer 2022). Ankur received the best Ph.D. thesis award in 2014, GIS-clinical partnership award in 2019, ASCO Merit Award in 2019, and UBC Supervisor Award in 2019. At Perkins, his team is working towards uncovering clinical implications of oncofetal ecosystem in therapeutic stratification.

Oncofetal ecosystem in liver cancer: spatial localisation and clinical implications

Oz Single Cell

Abstract Selected Oral

Uncovering context-specific and dynamic genetic regulation of gene expression in PBMCs at single-cell resolution

Anna S.E. Cuomo[1,2], Daniel G. MacArthur[1,2], Joseph E. Powell[1,3]

[1]Garvan Institute of Medical Research, Sydney, Australia
[2]Centre for Population Genomics, Garvan Institute of Medical Research, Sydney, NSW and Murdoch's Children Research Institute, Melbourne, Victoria, Australia
[3]UNSW Cellular Genomics Futures Institute, University of New South Wales, Sydney, NSW, Australia

Single-cell RNA sequencing (scRNA-seq) is widely applied to assess cellular heterogeneity in human tissues and cell-based models. Technological advances and exponential reduction in cost have enabled the first population-scale scRNA-seq studies, which have assayed single-cell transcriptomes in hundreds of genetically diverse individuals.

However, current workflows to analyse these data remain primarily based on principles for analysing conventional bulk expression quantitative trait locus (eQTL) studies, and hence fail to fully exploit complex scRNA-seq readouts. A critical limitation of existing approaches is the need to define discrete cell types for eQTL mapping a priori, which limits novel opportunities to chart continuous and unbiased landscapes of regulatory variants.

To address this, we recently proposed CellRegMap, a statistical framework to map regulatory variants across the continuous manifold of cellular environments estimated from scRNA-seq. CellRegMap allows for testing and characterisation of genetic effects on gene expression at the resolution of individual cells while flexibly sharing statistical strength related to cell states. Our framework provides a principled strategy to identify and characterise heterogeneous genetic effects that vary across cell states and cell types.

Here, we applied CellRegMap to single-cell data from peripheral blood cells from around one thousand individuals, identifying context-specific and dynamic eQTLs in blood at unprecedented resolution. Additionally, we used matched whole-genome sequencing (WGS) data from all thousand individuals to call rare variants, and extended our framework to test for effects of those variants on single-cell expression, leveraging an efficient implementation of kernel-based gene-set association testing. Finally, we identified genetic regulation that varies across sex and age of the individual assessed.

Comparison of haematopoietic cells across vertebrates using single cell transcriptomes

Carolyn de Graaf [1], Liam Salleh [1], Christine Biben [1], AnneMarie Welch [1], Douglas Hilton [1]

[1] *The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia*

The major hematopoietic lineages have existed since early in vertebrate evolution, covering the key functions of oxygen transport, clotting and defense against infection. Some of the details of how these functions are performed are unique to mammals, such as using platelets formed from polyploid megakaryocytes for clotting rather than thrombocytes.

Using single cell transcriptomes, we have created an atlas of gene expression of haematopoietic cells in mammals, chickens and fish, and annotated cells to their closest mammalian homologue by lineage. This has allowed us to look at how conserved haematopoietic gene expression is in different vertebrates in relation to human blood cells.

We have identified genes which are conserved as signatures of blood and immune lineages across all vertebrates and genes which are only associated with mammalian haematopoiesis.

We have then taken the mammalian specific and vertebrate conserved gene lists and looked at their regulation via ATAC-seq, and found that the more broadly conserved genes have more lineage specific open chromatin regions associated with them than genes with mammalian specific gene expression. This suggests that redundancy in regulation helps maintain lineage specific expression in divergent species.

Invited Speaker

Delphine Merino

Olivia Newton-John Cancer Research Institute

A/Prof Delphine Merino received her PhD from the University of Burgundy (France), investigating the role of the extrinsic pathway of apoptosis in cancer cell survival and drug resistance. In 2008, she joined the Molecular Genetics of Cancer Division at WEHI (Australia) as postdoctoral fellow to study the function of the intrinsic survival pathway in immune homeostasis and cancer progression. Her work was extended to the study of BH3 mimetics, small molecules that inhibit pro-survival proteins. She tested the impact of BH3 mimetics on normal and malignant lymphoid cells. In 2012, she joined the Stem Cells and Cancer Division (WEHI) as senior post-doctoral fellow, to study breast cancer progression and the effect of BH3 mimetics on several breast cancer subtypes.

Since 2017, Merino is a laboratory head at the Olivia Newton-John Cancer Research Institute (Australia). Her group focuses on tumour progression and heterogeneity. Her aim is to identify the biological features of the cells which are most likely to resist standard therapy and form clinically relevant macro-metastasis. Her lab uses patient derived xenografts, imaging, single cell sequencing and cellular barcoding to understand how these cells survive in different organs and interact with their microenvironment. These models will be useful not only to understand how the tumour spreads, but also to identify new therapies for the treatment of advanced disease.

Single cells: their mother/daughter relationships and interactions with neighbours revealed by cellular tracking

The development of novel single cells technologies has uncovered the extent of intra-tumoral heterogeneity in many types of cancer, including breast cancer. Several tumour clones present in primary tumours may contribute to metastasis and drug resistance. To understand the cellular and molecular mechanisms involved in tumour progression, we are using single cell sequencing to study the heterogeneity of breast cancer patient samples, and optical or genetic barcoding to further track the fate of individual human cancer cells and their daughters in different tumour microenvironments. These preclinical models allow us to study the spatio-temporal evolution of multiple cancer clones in different conditions, taking into consideration the clonal origin of the cells, their interaction under the influence of normal cells from the ‘neighbourhood’, and the impact of drug exposure on their fitness.

Invited Speaker

Geoffrey McDermott

Science and Technology Advisor, 10x Genomics, Australia

Geoff has spent his career working at the crossroads of chemistry, biology, and microfluidics to help drive early-stage research concepts into successful scientific ventures. He earned his BSc in Forensic Science and his PhD in Chemistry from Deakin University, Australia. Geoff is an early-stage employee of 10x Genomics and is currently a Science and Technology Advisor based in Australia. Prior to this role, he held a senior position within R&D at 10x Genomics, where he was part of the product development team commercializing single cell products.

Accessing new sample types to expand your research: Single Cell Fixed RNA Profiling and Visium CytAssist

Join us to learn how the new Chromium Single Cell Fixed RNA Profiling assay and Visium CytAssist instrument can help you push the boundaries of your research. The Fixed RNA Profiling assay enables storage for transport and/or batched runs in single cell experiments with PFA fixation, without compromising sample integrity or data quality. The Visium CytAssist enables spatial profiling insights to be gained from even more samples by facilitating the transfer of transcriptomic and proteomic analytes from standard glass slides to Visium slide

Invited Speaker

Nathan Palpant

Institute for Molecular Bioscience, the University of Queensland Australia

Associate Professor Nathan Palpant is a Group Leader at the University of Queensland's Institute for Molecular Bioscience. Dr Palpant is recipient of the International Society for Heart Research Young Investigator Award, the Lorne Genome Millennium Science Award, and a Heart Foundation Future Leader Fellowship. He is on the leadership of regional and national research programs including as co-chair of the Queensland Cardiovascular Research Network. His laboratory uses multi-scale experimental approaches including genomics, stem cell biology, and animal physiology to study mechanisms of cardiovascular development and disease. He is scientific co-founder of Infensa Bioscience, aiming to advance novel therapeutics for ischaemic cardiovascular diseases. His research has been featured in a documentary on the ABC TV program Catalyst, and major news outlets including The Guardian, Newsweek, The Washington Post, and The New York Times.

Unsupervised methods to determine cell-cell and gene-gene relationships

Cell barcoding system are enabling multiplexed single-cell RNA sequencing for high throughput perturbation of customisable and versatile experimental conditions. Unfortunately, most data are enriched in housekeeping and structural information, while regulatory gene programs are lowly expressed and difficult to identify. To address this, we developed a new computational analysis pipeline that overcomes limitations of mathematically-defined algorithms by using an unsupervised, genome-wide, orthogonal biological reference point to reveal the cell diversity and regulatory networks of any input data set. Collectively, these methods provide a cellular and computational pipeline to study cell differentiation applicable to diverse fields of developmental biology, drug discovery, and disease modelling.

Oz Single Cell

Abstract Selected Oral

LoxCode barcoding resolves maintenance of the long-term haematopoietic stem cell pool through symmetric self-renewal

Sara Tomei[1], Tom Weber[1], Shalin H. Naik[1]

[1]Immunology Division, The Walter & Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

In classical models of haematopoiesis, a pool of self-renewing long-term hematopoietic stem cells (LT-HSC) gives rise to all blood cell lineages. This self-renewal could occur through two non-mutually exclusive possibilities; 1) the HSC divides symmetrically giving rise to two identical daughter cells that both either remain in the pool or later differentiate; 2) HSCs that divide asymmetrically giving rise to one stem cell, and one differentiating cell. Both models have been demonstrated in vitro but it is still unclear how HSCs maintain the stem cell pool and contribute to the blood system at a clonal level in a native unperturbed in vivo setting.

In this work, we use a novel in situ barcoding system, termed the Loxcode mouse, to investigate LT-HSC self-renewal and contribution to native haematopoiesis. We LoxCode label mouse LT-HSC and track their clonal relationships to other downstream stem and progenitor cell types over multiple timepoints (2 weeks – 32 weeks). As expected, we found that the percentage of barcodes shared between LT-HSC and downstream progenitors increased with time, indicating that LT-HSC are exiting quiescence, dividing and that at least a few daughters of the original cell differentiate. However, we also found barcodes represented in multiple LT-HSCs that were dividing to maintain the LT-HSC pool. Surprisingly, these symmetrically dividing LT-HSCs were not contributing to downstream progenitors and therefore were not a major contributor to native haematopoiesis. Instead, we found that most LT-HSC barcodes contributing to haematopoiesis were separate and of low frequency i.e. little indication of symmetric self-renewal, and consistent with asymmetrical self-renewal.

Taken together this data suggests that there are at least two distinct types of dividing LT-HSC; one with the principal role of producing other LT-HSC and therefore maintaining the stem cell pool, while the other with the main role of producing mature blood lineages and maintaining the haematopoietic system.

Invited Speaker

Seyhan Yazar

Garvan Institute of Medical Research

Seyhan Yazar received a Bachelor Medical Science from the University of New South Wales, a Masters in Orthoptics from the University of Sydney and a PhD in Ophthalmic Genetics and Epidemiology from the University of Western Australia. In her thesis, she investigated the genetic and environmental influences on ocular disease development and progression with a particular interest in myopia, corneal dystrophies and glaucoma through exploring datasets from large population-based studies including the Western Australian Pregnancy Cohort (Raine) Study.

Recently, she has been awarded with a NHMRC CJ Martin Overseas Biomedical Fellowship to undertake further research training in the UK. Her current research interests include computer programming, statistics, engineering sequence analysis pipelines and performing well-validated and reproducible computational eye research.

Single-cell eQTL mapping identifies cell type-specific genetic control of autoimmune disease

The human immune displays substantial variation between individuals, defining how people vary in susceptibility to disease and respond to pathogens or cancer. Our knowledge of how genetic differences contribute to immune variation at the cellular level has been limited by the ability to generate sequence data from large numbers of cells and from enough individuals to test the relationship between genetic variation and cellular phenotypes. To address this, we developed the OneK1K cohort, consisting of single-cell RNA sequencing (scRNA-seq) data from 1.27 million peripheral blood mononuclear cells collected from 982 donors. We developed a framework for the classification of individual cells. By combining the scRNA-seq data with genotype data, we mapped the genetic effects on gene expression in each of 14 immune cell types and identified 26,597 independent cis–expression quantitative trait loci (eQTLs). We show that most of these have an allelic effect on gene expression that is cell type-specific. We show how eQTLs have dynamic allelic effects in B cells transitioning from naïve to memory states, identifying 333 eQTL whose allelic effects change as cells move across the B cell maturation landscape. Finally, we integrated genetic association data for seven common autoimmune diseases. We uncovered the causal route by which 305 loci contribute to autoimmune disease through changes in gene expression in specific cell types and subsets. Our work combines population genetics and scRNA-seq data to uncover drivers of interindividual variation in the immune system. Understanding the genetic underpinnings of immune system regulation will have broad implications in treating autoimmune diseases and infections, transplantation, and cancers.

Invited Speaker

Longqi Liu

BGI Research & Chinese Academy of Sciences

Dr. Longqi Liu is one of the chief scientists on sing-cell and spatial omics at BGI·Research. He obtained his PhD research at Chinese Academy of Sciences. There he studied the epigenetic mechanism underlying somatic cell reprogramming to pluripotency. He then moved to BGI·Research as a group leader, focusing on development of single-cell multi-omics technologies. He has won the first prize of the Natural Science of Guangdong Province, the first prize of the Chinese Postdoctoral Science Foundation, and the Dean's Excellence Award of the Chinese Academy of Sciences. Dr Liu leaded the development of DNBelab C4 high-throughput single cell microfluidic system, mapped the first cell atlas of non-human primate (Nature, 2022), developed spatial transcriptomic platform Stereo-seq and mapped the Spatiotemporal transcriptomic atlas of embryogenesis in mouse, fly and zebrafish (Cell, 2022, Developmental Cell, 2022[a], Developmental Cell, 2022[b]). He is also one of Founding Member of the SpatioTemporal Omics Consortium (STOC), which has attracted 100+ members from over 20 countries so far.

High-resolution panoramic spatial transcriptomics using DNA nanoball patterned arrays.

Oz Single Cell

Abstract Selected Oral

Proliferation is a driver of quorum sensing in an in vitro model of tissue resident macrophages

Nadia Rajab[1], Linden J. Gearing[2,3], Yair D.J. Prawer[1], Paul W. Angel[1], Sean M. Grimmond[4], Andrew L. Laslett[5,6], Jarny Choi[1], Christine A. Wells[1]

[1]Centre for Stem Cell Systems, Department of Anatomy and Physiology, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, VIC, 3010, Australia
[2]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, 3168, Australia
[3]Department of Molecular and Translational Sciences, Monash University, Clayton, VIC, 3168, Australia
[4]University of Melbourne Centre for Cancer Research, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, VIC, 3010, Australia
[5]CSIRO Manufacturing, Clayton, VIC 3168, Australia
[6]Australian Regenerative Medicine Institute, Monash University, Melbourne, VIC 3800, Australia

Macrophages coordinate the initial host inflammatory response to tissue infection as well as mediating the reparative phase by producing growth factors that promote tissue repair. One model of this functional dichotomy is that peripherally recruited monocyte-derived macrophages drive acute inflammatory responses to infection, whereas tissue resident macrophages are responsible for tissue repair. Alternatively, inflammation and repair may be inter-dependent molecular programs, such that both recruited and resident cells have equivalent capacity to contribute. In this study, induced pluripotent stem cells and peripheral blood monocytes were used to generate models of tissue-resident and recruited macrophages, respectively. Using single-cell sequencing, we assessed the impact of lipopolysaccharide activation in a 'stem cell village' model of macrophage behaviour. The heterogeneity of responses observed in these two populations highlighted that proliferation of resident macrophages is a driver of quorum sensing in host responses to bacterial ligands. Recruited monocyte-derived macrophages have limited proliferative ability, and quickly 'tolerise' to repeated pathogen exposure. This work suggests a new model where both recruited and resident macrophages are important drivers of an acute inflammatory response, but proliferation of resident macrophages ensures subsets of inflammatory macrophages are available to fight persistent infection.

Invited Speaker

Anuja Sathe

Department of Medicine, Stanford University US

Dr. Anuja Sathe completed her training in Medicine (M.B.B.S.) at the Maharashtra University of Health Sciences, India, an MRes in Molecular Medicine at the University of Glasgow, UK, and a Ph.D. in Cancer Biology at the Technische Universitaet Muenchen, Germany. She then joined Stanford University as a Postdoctoral Researcher in the Division of Oncology, where she is currently a Research Scientist. Anuja's research interests are in understanding and overcoming resistance to treatment in cancer patients. Her focus is on heterogeneity in tumor subclones and the microenvironment. Her work involves using single-cell genomics and patient-derived models to identify new treatment targets and understand when and how they work.

Understanding heterogenous cellular responses to cancer immunotherapy

Oliver Braubach

Director of Applications, Akoya Biosciences

Dr. Braubach received his doctorate from Dalhousie University in 2011. He then completed postdoctoral appointments at Yale and the Korea Institute of Science and Technology. Oliver's most recent academic appointment was at Cedars-Sinai where he conducted research in line with his background in optical imaging. He has been leading Akoya's Applications development since 2017.

Discovering and Truly Multiomic Spatial Tissue Biology

Insights gathered from spatial proteomics and transcriptomics have provided massive contributions to tissue biology. Until recently, however, these experiments were part of distinct research agendas and data were rarely combined. Today, we seek a true spatial multiomic approach that integrates single-cell spatial proteomic data with single-cell spatial transcriptomics to provide a holistic view of tissue biology. Dr. Oliver Braubach will discuss how Akoya Biosciences' new multiomic solution redefines spatial phenotyping in discovery and translational research.

This talk is sponsored by our gold sponsor: Akoya/Geneworks

Invited Speaker

Jacky Li

Murdoch Children's Research Institute, Victoria, Australia

Jacky Li is a PhD student studying the development of human blood stem cells in Professor Andrew Elefanty's Blood Diseases and Development laboratory at Murdoch Children's Research Institute. This laboratory has a focus on using induced pluripotent stem cells used as a model for human fetal blood development. He is especially interested in the different RNA sequencing technologies and bioinformatics tools that can aid in this type of research.

Benchmarking of in vitro differentiated induced hematopoietic stem cells (iHSCs) against human embryonic HSCs

Human blood development occurs in several waves at different embryonic sites, regulated by time sensitive, complex signaling networks. To recapitulate haematopoiesis and the generation of haematopoietic stem cells in vitro, we have generated 'induced HSCs' (iHSCs) from human pluripotent stem cells (hPSCs) differentiated in a 'swirling' culture protocol. Single cell RNA-sequencing of iHSCs and the other haematopoietic and stromal components of the swirler culture enabled us to study the relationships between mesoderm derived endothelial progenitors, blood cells and stromal elements. Transcriptomics revealed extensive heterogeneity of early haematopoietic progenitors including lymphoid, myeloid and erythroid lineages. The transcriptional profiles of arterial, venous and hemogenic endothelial cells also resolved into several distinct clusters. Comparing the iHSCs with datasets of early human blood development, we found a high degree of similarity between in vivo and in vitro generated venous and arterial endothelial cells as well as hematopoietic stem and progenitor cells (HSPCs). Exploring the role of retinoids during hematopoietic differentiation, we found that retinoid treatment of hPSC differentiated cells upregulated key genes that were also expressed in the HSPC clusters within the human embryo datasets. In summary, single cell data of iHSCs, compared to human embryonic hematopoietic cells, illustrated the high fidelity of the in vitro differentiated cells. Some points of difference between the transcriptional profiles of hPSC-derived and human embryonic haematopoiesis were also identified, providing clues how to further improve differentiation protocols to achieve more efficient generation of repopulating iHSCs in vitro.

Oz Single Cell

Abstract Selected Oral

Multi-omic Profiling of Rogue Lymphocytes in Celiac Disease

Raymond Louie[1], Mandeep Singh[2], Thiruni Adikari[1], Matt Field[3], Ira Deveson[2], Timothy Amos[2], Jason Tye-Din[4], Golo Ahlensteil[5], Christopher Goodnow[2], Fabio Luciani[1]

[1]*University of New South Wales, Sydney, Australia*
[2]*Garvan Institute of Medical Research, Sydney, Australia*
[3]*James Cook University, Queensland, Australia*
[4]*Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia*
[5]*Western Sydney University, Sydney, Australia*

A major hurdle in understanding the immune pathogenesis of human autoimmune diseases is distinguishing self-reactive “rogue” lymphocytes from normal immune cells required for host defence. The environmental trigger (gluten) and major CD4+ T cell epitopes driving coeliac disease are well characterised, but the underlying mechanisms by which rogue lymphocytes cause coeliac disease are unknown. Here, we apply multi-omic technologies that enable detailed DNA, RNA and protein measurements at the single-cell level to profile tens of thousands of immune cells isolated from small intestinal biopsies from individuals with coeliac disease. We utilise the T-cell receptor as a natural barcode across multiple single-cell experiments to identify expanded rogue T cell clones and their gene and cell-surface protein expression profiles, along with any somatic DNA driver mutations. Strikingly, we identify in multiple patients expanded T cell clones with mRNA and cell-surface protein expression profiles of cytotoxic effector cells, that harboured missense somatic mutations in T cell lymphoma driver genes STAT3, STAT5B and DDX3X. These have been described as strong gain-of-function mutations in T cell lymphomas, highlighting a novel mechanism by which T cell clones escape immune tolerance and adopt a rogue phenotype in coeliac disease. Overall, our results highlight the power of single-cell multi-omics in identifying and characterising rare pathogenic clones in a common human autoimmune disease.

Oz Single Cell

The Grand Debate

The topic:

Data analysts are more important than data generators….

Affirmative Team

Tom Ashhurst

Jovana Maksimovic

Daniel Brown

Negative Team

Alicia Oshlack

Scott Berry

Melissa Davis

Hosted by
Shalin Naik

voted via clap-o-meter by YOU!

Oz Single Cell

Gala Dinner

Join us on the 77th floor in Gold Coast's highest banquet hall for our Oz Single Cell Gala Dinner!

At the Gala Dinner, we will be announcing the winners of the Illumina SkyPoint challenge, and also hear from co-founder of 10x Genomics, Ben Hindson.

Look forward to spectacular night time views, a two course meal, and a night filled with live music, networking (on the dance floor), and much, much more!

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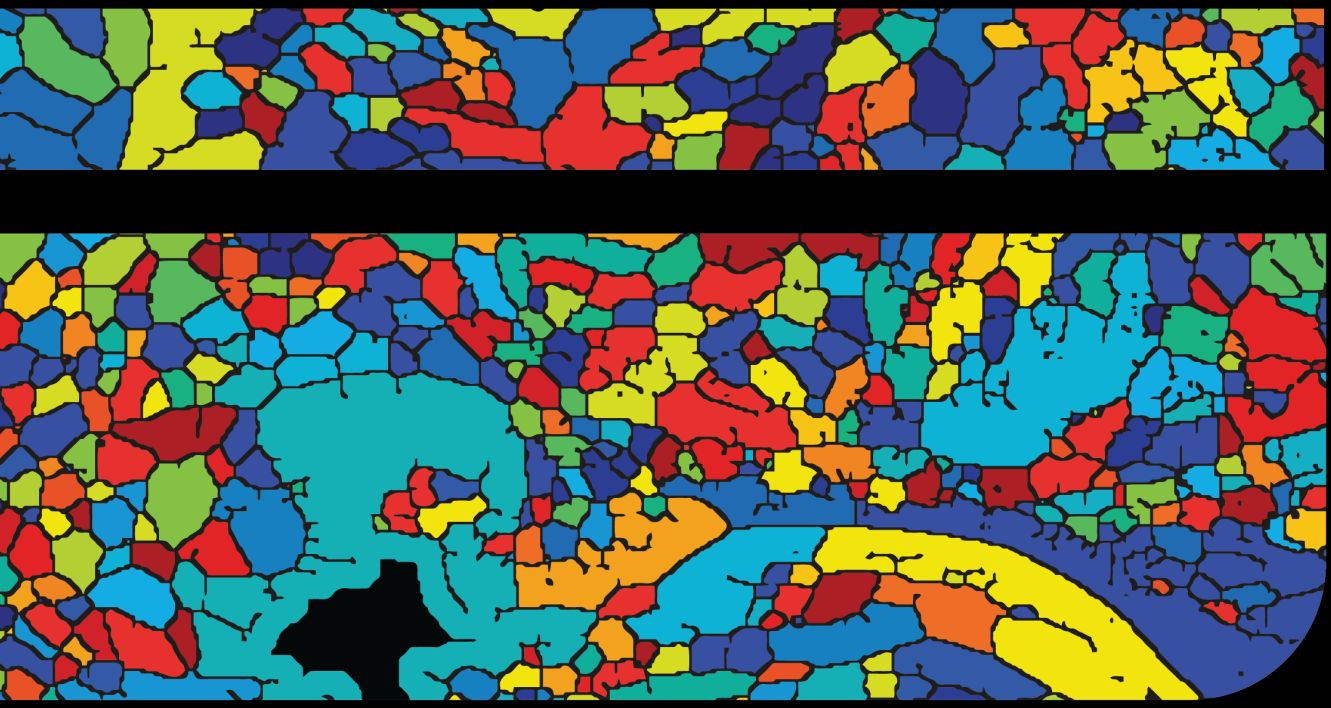
Day 3 Talks



Invited Speaker

Mohammad Lotfollahi

Helmholtz Zentrum München

Mo has recently finished his Ph.D. in computational biology at the lab of Fabian Theis and is an incoming faculty member at Welcome Sanger institute. During his Ph.D. he gained industry experience by interning in both AI research companies such as Facebook and also Pharma industry. Mo's research is interested in leveraging machine learning to understand health and disease using single-cell biology for drug discovery, cell engineering, and clinical applications.

Multi-scale learning on single-cell atlases: from cells to patients

Oz Single Cell

Abstract Selected Oral

Comparative spatial multiomics analyses of a cell atlas and interactome in skin cancer reveal cell types, gene markers, and cell-cell interactions underlying skin cancer diversity

Guiyan Ni [1], Laura Grice [1,2], Xinnan Jin [1], Minh Tran [1], Onkar Mulay [1], Emily E Killingbeck [3], Mark T Gregory [3], Siok Min Teok [1], Katharina Devitt [4], Arutha Kulasinghe [4], Michael Leon [3], Sarah Warren [3], Peter Soyer [4], Mitchell Stark [4], Kiarash Khosrotehrani [4], Ian Frazer [4], Youngmi Kim [3], Quan Nguyen [1]

[1] *Division of Genetics and Genomics, Institute for Molecular Bioscience, The University of Queensland, Australia*
[2] *School of Biomedical Sciences, The University of Queensland, Australia*
[3] *NanoString® Technologies , Seattle WA 98109, USA*
[4] *Diamantina Institute, The University of Queensland, Australia*

The three major skin cancer types - squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and melanoma - derive from the same outer skin layer but vary widely in cell type composition and interactions within the cancer microenvironment. These variations underlie differential cancer initiation, progression and outcomes. To identify the cells and oncogenic signalling pathways driving the initiation and progression of different skin cancers, a knowledge base of cancer cell type composition and cell-to-cell interactions is essential.

Here, we profiled and compared the cellular makeup and interactome of SCC, BCC and melanoma, by integrating six distinct yet complementary single-cell and spatial technologies. First, using single-cell RNA sequencing, we generated a skin cell atlas of >50,000 cells from 11 healthy and SCC skin biopsies. Unsupervised clustering identified 30 molecularly-distinct cell types, 18 of which were significantly enriched in SCC cancerous tissues. Interestingly, these cancer-associated subsets have been linked to pro-tumour signalling and immunological anti-tumour responses. We then mapped and validated spatial distribution and cell type localisation using Nanostring GeoMx, CosMx, and 10x Genomics Visium technologies. We further constructed a spatial cell-to-cell interaction atlas, screening 2293 (for Visium) and 517 (for CosMx) LR pairs at spot and single-cell level. We identified 19 LR pairs specific to individual skin cancer types, some of which have been previously implicated in skin cancer progression and metastasis, suggesting potential interactions associated with differences in metastasis potential between each of the three cancer types.

Overall, we have built two spatial atlases, a cell atlas of 30 cell types mapped to skin layers, and an interactions atlas with hundreds of thousands of possible interactions between these cell types. Such a resource is especially needed for skin cancer, accounting for over 70% of all cancer. We made the resources available through a web application accessible to the broad community.

A single-cell and spatially resolved atlas of human breast cancers

Daniel Roden [1,2], Sunny Wu [1], Ghamdan Al-Eryani [1,2], Beata Kiedik [1,2],, Simon Junankar [1], Kate Harvey [1], Aatish Thennavan [3], James Torpy [1], Nenad Bartonicek [1], Alma Andersson [4], Stephen Williams [5], Mun Hui [1,6], Sandra O'Toole [7], Elgene Lim [8], Joakim Lundeberg [4], Charles M. Perou [3], and Alexander Swarbrick [1,2]

[1] *The Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia*
[2] *School of Clinical Medicine, UNSW Sydney, NSW 2052, Australia*
[3] *Department of Genetics, University of North Carolina, Chapel Hill, NC, 27599, USA*
[4] *Science for Life Laboratory, Division of Gene Technology, KTH Royal Institute of Technology, Stockholm, Sweden*
[5] *10x Genomics, Pleasanton, CA, 94588, USA*
[6] *Chris O'Brien Lifehouse, Camperdown, New South Wales, Australia*
[7] *Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia*
[8] *St Vincent's Hospital, Darlinghurst, NSW 2010, Australia*

Breast cancers are complex cellular ecosystems where heterotypic interactions play central roles in disease progression and response to therapy. However, our knowledge of their cellular composition and organization remains limited.

Recently we published an integrated cellular and spatial atlas of 26 primary human breast cancers [1]spanning all major molecular subtypes, providing a systematic characterization of the cellular diversity of the epithelial, immune and stromal cellular landscape. To investigate neoplastic cell heterogeneity, we developed a single-cell classifier of intrinsic subtype (scSubtype) and revealed recurrent transcriptional gene modules that define the neoplastic cells. This detailed cellular taxonomy was then used to deconvolute large breast cancer cohorts, allowing their stratification into tumour 'ecotypes', with unique cellular compositions and clinical outcome. Further, we used Visium spatial profiling to provide an initial view of how stromal, immune and neoplastic cells are spatially organized in breast tumours, offering insights into tumour regulation.

This work is now being expanded with the generation and integration of single-cell profiles of 100s of breast cancers and matching datasets of spatially resolved transcriptomes. We propose that this will identify recurrent, spatially organized cellular niches in breast tumours, offering insights into anti-tumour immune regulation and neoplastic heterogeneity. This increased scale will also allow detailed characterisation of intra- and inter-subtype differences and the identification of associations between cellular, molecular features and clinicopathological features.

Our work highlights the potential of large-scale, integrated, cellular and spatial genomics to unravel the complex cellular heterogeneity within tumours and identify novel cell types, niches, and regulatory states that will inform treatment response.

Oz Single Cell

Abstract Selected Oral

Brain cell atlas of an anhydrobiotic insect: cell-type-specific mechanisms of complete desiccation tolerance and insights for new neuroprotectors for treatment of reperfusion syndrome

Oleg Gusev [1,2], Alexander Dekan [2], Alexander Nesmelov [2], Takahiro Kikawada [3], Richard Cornette [3], Elena Shagimardanova [2]

[1] Graduate School of Medicine, Juntendo University, Tokyo, Japan
[2] Regulatory Genomics Research Center, KFU, Kazan, Russia,
[3] National Agriculture and Food Research Organization, Tsukuba, Japan

Larvae of the sleeping chironomid Polypedilum vanderplanki (Diptera) are the only insects able to withstand complete desiccation by induction of a reversible ametabolic state – anhydrobiosis. Anhydrobiotic larvae lose more than 96% of water in the body and all of biological activity stops. Tolerance of brains of the larvae is a unique model for the search for new neuroprotectors, as it experiences a ‘super stroke’ associated with long anoxia and a burst of oxidative stress upon rehydration and then recovers completely functionally in less than one hour of rehydration.

We have recently shown that the ability to tolerate complete desiccation in the sleeping chironomid is based on the drastic paralogous expansion of unique protective gene groups (Yoshida et.al, 2022; Gusev et.al 2014). Here, we report the creation of a snRNAseq atlas of the brain of the sleeping chironomid. The expression profile 50 000+ brain nuclei reflects different stages of the dehydration-rehydration cycle of the larvae. It is the first complex study of anhydrobiosis on a single-cell resolution in eukaryotes.

We found that during the cycle of complete desiccation – rehydration, the brain's cell composition experiences significant remodeling. We also found that the protection of the brain against the harmful effect of desiccation largely relies on cell type-specific transcriptional activity of the multiple-members protective gene families and there are uniquely combined protectors for glia and other cell types.

Finally, by a combination of enriched molecular pathways in brain cells and metabolic profiles of larvae, we identified new potential neuroprotectors that ensure the survival of different cell types in the brain in a “super-stroke” anhydrobiotic state.

Invited Speaker

Hamish King

Walter and Eliza Hall Institute of Medical Research

Hamish King completed his undergraduate and Honours at Flinders University in Adelaide, before moving to the United Kingdom to undertake his PhD training in molecular genetics at the University of Oxford with Prof Rob Klose. While there he studied how gene expression is regulated by chromatin-modifying complexes, and how sequence-specific transcription factors cooperate with chromatin remodellers to access and bind the genome.

Following his PhD, Hamish was a Sir Henry Wellcome Postdoctoral Fellow in the lab of Dr Louisa James at the Blizard Institute, Queen Mary University of London, where he studied the transcriptional and epigenetic regulatory networks that determine B cell identity and function in the human immune system. As part of this fellowship, Hamish worked closely with Dr Sarah Teichmann (Wellcome Sanger Institute, Cambridge) and Prof William Greenleaf (Stanford). Building on his recent discoveries, Hamish joined the WEHI as a Laboratory Head in February 2022, where he is focused on understanding the molecular mechanisms that underpin global and locus-specific epigenetic regulation of gene expression in human B cell maturation. The long term goal of his lab to understand how these processes go awry in disease so that new treatments and diagnostic tools can be developed to improve patient outcomes and quality of life.

Single cell genomics to map human B cell maturation and etiology of autoimmune risk loci

B cell-mediated immune responses and memory form in secondary lymphoid organs and form a major arm of the adaptive immune system to fight and remember infections. During this process, B cells undergo affinity maturation in the germinal centre reaction and errors at this stage can lead to either defective immune responses or contribute to autoimmune disease. Many questions remain about the dynamic cellular states involved in both normal and disease-related B cell maturation, including the gene regulatory networks that underlie key cell fate decisions and phenotypes. We have generated a comprehensive roadmap of human B cell maturation by defining the gene expression, antibody repertoires, and chromatin accessibility of diverse B cell states at single-cell resolution. We use this roadmap to reconstruct gene expression and transcription factor dynamics during B cell activation and to interpret potential regulatory impact of genetic variants implicated in autoimmunity. We predict that many autoimmune-linked GWAS variants exhibit their greatest regulatory potential in germinal centre-associated cell populations, providing new insights into the cellular and genetic causes that may underpin autoimmune disease.

Invited Speaker

Alistair Forrest

Harry Perkins Institute of Medical Research

Professor Forrest BSc(Hons), MIT, PhD was born in Western Australia and obtained his BSc (Hons) in Biotechnology at Murdoch University in 1993. He then moved to Brisbane and while working as a research assistant at the QIMR completed a Masters in Information Technology at the Queensland University of Technology. Shortly afterward, he completed his PhD in Bioinformatics at the Institute for Molecular Bioscience at the University of Queensland. During his time in Brisbane he was involved in both 'wet' and 'dry' science, generating and analysing some of the first microarrays used in Australia and invented a strand specific RNA-seq protocol that heralded the start of the RNA-seq revolution. In 2007 he moved to RIKEN Yokohama Japan on a CJ Martin Fellowship. Over the past seven years he has progressively been promoted at RIKEN taking on more senior roles, and is currently scientific coordinator of the FANTOM5 (Functional Annotation of the mammalian genome) project consisting of a consortium of over 250 scientists in 20 countries. This has used single-molecule sequencing to generate a map of promoters and enhancers across a large collection of human and mouse primary cells, cancer cell lines and tissues. The work has recently published in the prestigious journal Nature along with a collection of 18 additional satellite papers in specialized journals such as Nature Biotechnology, Genome Research and Blood.

After 20 years away from Perth he returned thanks to funds raised through the MACA Cancer 200 and a Cancer Research Trust Senior Cancer Research Fellowship.

A cellular and spatial atlas of mammalian tongue

The mammalian tongue epithelium is a complex tissue with specialised structures including filiform papillae, which have a role in both mastication and mechanosensation, and fungiform, circumvallate and foliate papillae which contain taste cells. Maintenance of the epithelium requires a constant process of renewal and regeneration of these structures. Here we applied single nuclei RNA-seq (snRNA-seq) to identify 15 major cellular populations within the murine dorsal tongue mucosa including various epithelial populations, skeletal muscle cells, immune, endothelial, schwann and taste cell populations. Sub clustering further split these into 44 subpopulations, including 15 epithelial subpopulations covering a continuum of basal, suprabasal and terminally differentiated apical cells. Using BGI STomics and Vizgen MERFISH spatial transcriptomics technologies to place each cellular population within a spatial context revealed the exquisite structure of the tongue and confirmed several transient epithelial populations predicted along trajectories of differentiation (anterior column, posterior column, and interpapillary). Finally, analysis of receptor and ligand expression patterns in the epithelial populations revealed complex cell-to-cell communication signalling both between cells that give rise to different epithelial structures and between cells at different stages of the same developmental trajectory. This study provides a systematic and unbiased survey of the cellular organisation of the adult mouse tongue mucosa.

Oz Single Cell

Abstract Selected Oral

The mutational landscape of single neurons and oligodendrocytes reveals evidence of inflammation-associated DNA damage in multiple sclerosis

Justin P. Rubio[1], Allan Motyer[2,3,4], Stacey Jackson[1], Bicheng Yang[5], Ivon Harliwong[5], Wei Tian[5], Wingin Shiu[6], Yunchang Shao[7,8], Bo Wang[7], Catriona McLean[9], Michael Barnett[10,11], Trevor J. Kilpatrick[1,12,13], Stephen Leslie[2,3,4]

[1]*The Florey Institute of Neuroscience and Mental Health, Victoria, Australia*
[2]*Melbourne Integrative Genomics, The University of Melbourne, Victoria, Australia*
[3]*School of Mathematics and Statistics, The University of Melbourne, Victoria, Australia*
[4]*School of BioSciences, The University of Melbourne, Victoria, Australia*
[5]*MGI-Australia, 300 Herston Rd, Herston, Queensland, Australia*
[6]*BGI-Australia, Herston, Queensland, Australia*
[7]*China National GeneBank, Jinsha Road, Shenzhen, China*
[8]*State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macao, Macao, China*
[9]*Victorian Melanoma Service, Alfred Health, Melbourne, Victoria, Australia*
[10]*Brain and Mind Centre, The University of Sydney, NSW, Australia*
[11]*Sydney Neuroimaging Analysis Centre, Camperdown, NSW, Australia*
[12]*Florey Department of Neuroscience and Mental Health, The University of Melbourne, Victoria, Australia*
[13]*Department of Neurology, Royal Melbourne Hospital, Victoria, Australia.*

Background. Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system, affecting 3 million people globally. In MS, autoreactive immune cells infiltrate the brain, causing injury to oligodendrocytes (OLs) and secondarily also neurons. The cascade of events that follow are believed to underpin progressive MS, the untreatable end stage of this debilitating disease.

In the current study, we set out to determine whether inflammation in the MS brain could be causing damage to the genomes of neurons and OLs, thus acting as a mechanistic link between neuroinflammation and neurodegeneration in MS.

Results. We isolated 172 single neuronal and OL nuclei from the post-mortem donor brain tissue of five MS cases and three neurotypical controls using fluorescence-activated nuclei sorting (FANS). This was followed by single nucleus whole genome sequencing (snWGS), from which somatic single nucleotide variants (sSNVs) were detected, and mutational signatures investigated.

For two of five MS cases, we identified a significant sSNV excess in neurons and OLs from pathology-affected tissue. For a case with primary progressive MS, this translated to a near 50-year increase in biological age of lesion resident cells. Mutational signature analysis conducted on all cells revealed an age-associated signature and a novel disease-specific signature that may shed light on the mutagenic mechanisms affecting neurons and OLs in MS.

Conclusions. We have conducted the first snWGS study for MS and the first comparison of the mutational landscapes of neurons and OLs in health or disease. Our results provide evidence that inflammation in the MS brain is associated with DNA damage in a subset of cases, and that a novel aggregation of mutational processes are involved.

Oz Single Cell

Abstract Selected Oral

An integrated cell barcoding and computational analysis pipeline for scalable analysis of differentiation at single-cell resolution

Sophie Shen[1], Tessa Werner[1], Yuliangzi Sun[1], Woo Jun Shim[1], Samuel Lukowski[1,2], Stacey Andersen[1], Han Sheng Chiu[1], Dalia Mizikovsky[1], Di Xia[1,3], Xiaoli Chen[1], Duy Pham[1], Zezhuo Su[4,5], Joseph Powell[6,7], Mikael Bodén[8], Joshua W. K. Ho[4,5], Quan Nguyen[1], Nathan J. Palpant[1]

[1] *Institute for Molecular Bioscience, Brisbane, QLD, Australia;*
[2] *Boehringer Ingelheim, Vienna, Austria;*
[3] *Genome Innovation Hub, University of Queensland, Brisbane, QLD, Australia;*
[4] *School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, Pokfuoam, Hong Kong SAR, China;*
[5] *Laboratory of Data Discovery for Health Limited (D²4H), Hong Kong Science Park, Hong Kong SAR, China;*
[6] *Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Sydney, NSW, Australia;*
[7] *University of New South Wales Cellular Genomics Futures Institutes, Sydney, New South Wales, Australia;*
[8] *School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia*

Single-cell technologies have elevated knowledge gain to new heights, though current workflows limit the possible scope of experimental designs and uncovering meaningful biology from noise remains a challenge. To address this, we have developed a versatile single cell multiplexing and data analysis platform to accelerate knowledge gain into mechanisms of cellular differentiation.

Using CRISPR gene editing, we engineered a cell barcoding system in isogenic human induced pluripotent stem cells to facilitate multiplexed single-cell RNA-seq experimental design and cell capture. Following this technology that upscales the possible rate of data generation, we develop a complementary computational analysis pipeline for unsupervised interpretation of cellular diversity in large scRNA-seq datasets. By summarising repressive H3K27me3 deposition patterns across 111 diverse cell types in the NIH Epigenome Roadmap dataset, we derive an orthogonal biological grounding point to identify the most well defined cell types in a dataset. These shared methylation patterns across cell types can then also be used to infer epigenetically co-regulated gene modules that reveal the functional and regulatory basis of each cell type.

We implement this integrated pipeline to generate a highly resolved and diverse dataset mesendoderm differentiation from induced pluripotent stem cells. Our cellular multiplexing allowed us to capture 62 independent experimental samples comprising 8 differentiation time points and 9 developmentally relevant signalling perturbations with duplicates, capturing 62,208 cells in total. We employ our analysis pipeline to deconstruct the temporal and gene regulatory basis of this multilineage stem cell differentiation, identifying and annotating 48 distinct cell types spanning ectoderm, mesoderm, and endoderm cell types. Overall, this study provides a cellular and computational pipeline and dataset to study cell differentiation applicable to diverse fields of developmental biology, drug discovery, and disease modelling.

Invited Speaker

Catherine Wong

Peking Union Medical College Hospital (Dongdan campus), Chinese Academy of Medical Science & Peking Union Medical College

Professor Catherine C.L. Wong is the Director of Center for Precision Medicine Multi-omics Research (CPMMR), Associate Professor with Tenure at School of Basic Medical Sciences, Peking University Health Science Center, Principal Investigator at Peking University-Tsinghua University Center for Life Sciences (CLS), and Honorary Professor at the University of Manchester.

Professor Wong's work is dedicated to the development of cutting-edge mass spectrometry-based proteomics technologies and their applications in basic biological and biomedical research. She identified beta-actin as the substrate of arginylation in vivo which greatly influenced the research field of this post translational modification (Science, 2006). The method of gOAD Chip (3.0), a single-cell proteome pico-chip currently developed in her lab, was recognized at the International Conference of Single-cell Proteome in both 2019 and 2020 as the method for identifying the highest amount of proteins in one cell. More recently, she developed an absolute quantitative proteomics method on mass spectrometry (TIMLAQ-MS) which lead to a discovery of new functions of TCR-CD3 complex (Cell, 2020), as well as used 4D-DIA high-throughput technology to reveal a “two-stage” mechanism of COVID-19 (Nat Commun, 2020). She also focuses on discovering authentic disease biomarkers and unveiling disease mechanisms based on large clinical cohorts. Professor Wong has published over 80 articles in the journals such as Cell, Science, Nature Methods and Nature Communications. In 2014, she was a recipient of the “Introducing Outstanding Technical Talents" (Technology Hundred Persons Plan) of the Chinese Academy of Sciences.

New version of Glass-Oil-Air-Droplet (GOAD) nano chip device and some applications

Deep single-cell proteome has great biological significance for understanding cellular heterogeneity. To overcome the barriers along the processes of shotgun SCP, we developed a nanoliterscaled glass-oil-air-droplet (GOAD 3.0) chip using miniaturized stationary droplet micro reaction and manipulation techniques for multi-step sample pretreatment. Compared to previous system, GOAD 3.0 system used integrated chip with the advantages of robust, corrosion resistance, high and low temperature resistance and reusable, substantially lowering hands-on time and sample evaporation. We identified the most of 2639 protein groups from single HeLa cell using timsTOF mass spectrometer in parallel-accumulation-serial-fragmentation (PASEF) scanning mode, and identify proteins over the complete range of less than 100 to more than 10 000 000 pc/c, achieving a dynamic range of 7 orders. We further demonstrate the application of GOAD chip device for the first time SCP analysis on embryo development and RNA-binding proteins. This method has great potential for achieving ultrasensitive proteomic analysis on single-cell level.

Speaker

Scott Berry

School of Medical Sciences, University of New South Wales, Australia

I lead a research group investigating quantitative control of gene expression at the single-cell level – ranging from epigenetic memory and cellular decision-making to global RNA metabolism in the context of cellular physiology.

Proper control of gene expression underpins the normal development of organisms, and is dysregulated in diseases such as cancer. We seek to better understand which components of a cell are involved in this control, and how they work together. Our fundamental discoveries regarding the normal operation of cells underpin the identification of new disease mechanisms and possible therapeutic targets. We also contribute to the development of new research methodologies and analysis methods for use in drug discovery and diagnostics applications.

We make extensive use of high-throughput microscopy, including highly multiplexed immunofluorescence and RNA fluorescence in situ hybridisation, as well as live-cell imaging and automated image analysis. Together, these approaches enable detailed measurement of quantitative cellular phenotypes (for example, the abundance and localisations of specific proteins or RNAs) across large cell populations. We analyse resulting datasets using data science methods and interpret and explore hypotheses through the development of minimal mathematical models. We complement these image-based experimental approaches with functional genomics experiments (based on next-generation sequencing) and we use perturbation experiments including genome and epigenome-editing to test specific hypotheses.

Multiplexed protein imaging from the subcellular to the cell-population scale

Highly multiplexed protein imaging enables localisation and quantification of tens to hundreds of different molecular species, with a spatial resolution approaching the diffraction limit of light. As an image-based method, it can be extended in a cost-effective manner to a scale of millimetres, which allows one to study subcellular, cellular, and population-scale phenotypes in the same assay. Here, I will discuss how we are using this technology in a high-throughput screening format to investigate mechanisms of cellular mRNA concentration homeostasis, and to characterise changes in subcellular organisation across heterogeneous cell states and perturbation conditions.

Speaker

Amrita Roy

Product Manager (IN, SEA, ANZ), Bio-Techne

From Seq to Spatial Insight: Interrogate novel cell phenotypes and spatial RNA signatures at single-cell resolution with gold-standard RNAscope technology

Characterizing the transcriptomic profiles of individual cells by single-cell RNA sequencing (scRNA-seq) has become a universal tool to identify both known and novel cell populations, ushering in a new era of single cell biology. This has proven to be especially true in complex organs with high cellular heterogeneity, such as the mammalian brain, embryos and tumors. However, scRNA-seq utilizes dissociated cells and results in the loss of spatial organization of the cell population being analyzed.

The RNAscope™ Multiplex Fluorescent assay, with simultaneous detection of up to 4 targets, and the new RNAscope HiPlex assay, with simultaneous detection of up to 12 targets in fresh frozen tissue section, provide pivotal single cell imaging data to confirm and spatially map gene profiles identified by scRNA-seq in complex tissues. Visualization of cellular gene expression with tissue morphology can provide greater insights into the function of cells and tissues.

Learn about:

1. RNAscope applications in Neuroscience, Oncology, Immunology, Developmental biology, Cell and Gene therapy and more.
2. Multiomic analysis using RNA-protein co-detection enabling simultaneous visualization of RNA biomarkers with cell-specific protein markers.
3. Gain valuable insights into disease pathology by uncovering novel molecular pathways by multiplex detection of target markers
4. Visualize the most diverse range of RNA biomarkers from mRNA to miRNA

This talk is sponsored by our gold sponsor: In Vitro Technologyes/ACDBio

Spatial transcriptomic maps of whole mouse embryos

Luyi Tian[1†], Abhishek Sampath Kumar[2†], Adriano Bolondi[2,3†], Amèlia Aragonés Hernández[2,3], Robert Stickels[1,4], Maria Walther[2], Leah Haut[2,3], Evan Murray[1], Lars Wittler[5], Helene Kretzmer[2], Evan Macosko[1,6*], Léo Guignard[7*], Fei Chen[1,8*], Alexander Meissner[1,2,3,8*]

[1] *Broad Institute of MIT and Harvard; 02142 Cambridge, MA, USA.*
[2] *Department of Genome Regulation, Max Planck Institute for Molecular Genetics; 14195 Berlin, Germany.*
[3] *Institute of Chemistry and Biochemistry, Freie Universität Berlin;14195 Berlin, Germany.*
[4] *Graduate School of Arts and Sciences, Harvard University; 02138 Cambridge, MA, USA.*
[5] *Department of Developmental Genetics, Max Planck Institute for Molecular Genetics; 14195 Berlin, Germany.*
[6] *Department of Psychiatry, Massachusetts General Hospital; 02114 Boston, MA, USA.*
[7] *Aix Marseille University, Toulon University, CNRS, LIS 7020, Turing Centre for living systems; 13000 Marseille, France.*
[8] *Department of Stem Cell and Regenerative Biology, Harvard University; 02138 Cambridge, MA, USA.*
[†] *These authors contributed equally to this work.*
** Lead contacts*

Precise spatiotemporal orchestration of gene expression is required for proper embryonic development. The application of high throughput single-cell technologies has provided comprehensive transcriptomic definitions of cell states within the embryo, but our knowledge of their spatial localization remains too limited. To address this and explore early mouse organogenesis, we used Slide-seq to generate high-resolution maps of E9.5 together with whole E8.5 and E9.0 mouse embryos built with sc3D, a tool for creating and exploring a three-dimensional (3D) 'digital embryo'. Our transcriptomic maps enabled the assessment of gene expression patterns along the anteroposterior and dorsoventral axes, with a particular emphasis on neural tube development, and revealed several genes with distinct spatial patterns. Furthermore, we use spatial transcriptomics as a tool to profile molecular phenotype of perturbed embryo. We determined the transcriptional identity of ectopic neural tubes in *Tbx6* mutant embryos, abnormal cell states in Dnmt1 mutant embryos, and compared cell states between normal and synthetic embryo generated in vitro. Taken together, we present a framework for an experimental and computational toolkit and showcase its power by systematic spatiotemporal dissection of complex embryonic structures and mutant phenotypes, paving the way for a broader investigation including congenital and developmental abnormalities.

Speaker

Jean Yang

Faculty of Science, the University of Sydney, Australia

Professor Jean Yang is an applied statistician with expertise in statistical bioinformatics. She was awarded the 2015 Moran Medal in statistics from the Australian Academy of Science in recognition of her work on developing methods for molecular data arising in cutting edge biomedical research. Her research stands at the interface between medicine and methodology development and has centered on the development of methods and the application of statistics to problems in -omics and biomedical research. She has made contributions to the development of novel statistical methodology and software for the design and analysis of high-throughput biotechnological data including that from microarrays, mass spectrometry and next generation sequencing. Recently, much of her focus is on integration of multiple biotechnologies with clinical data to answer a variety of scientific questions. This includes developing various approaches and methodologies in statistical machine learning and network analysis. As a statistician who works in the bioinformatics area, she enjoys research in a collaborative environment, working closely with scientific investigators from diverse backgrounds.

Atlas-scale data integration for single-cell meta analysis

Speaker

Ryan Lister

Harry Perkins Institute of Medical Research

Professor Ryan Lister was elected 2020 Fellow of the Australian Academy of Science and joint Scientist of the Year in the 2020 Premier's Science Awards. He completed his Bachelor's degree in Biochemistry and Genetics at The University of Western Australia (UWA) in 2000, and undertook his PhD studies in plant mitochondrial biogenesis at UWA from 2001-2005. After completing his PhD, Ryan was awarded a Human Frontiers Science Program Postdoctoral Fellowship in 2006 to undertake his postdoctoral studies in Joseph Ecker's laboratory at The Salk Institute for Biological Studies in California, where his research focused on studying the epigenome, the molecular code superimposed upon the genome that plays important roles in regulating the information contained in the underlying DNA sequence. He developed new high-throughput DNA sequencing methodologies to profile the transcriptome and epigenome, including the first RNA-sequencing approach and whole genome bisulfite sequencing. This allowed, for the first time, the precise mapping of sites of DNA methylation throughout an entire genome, and he used these tools gain new insights into the epigenome in a variety of systems, including plants, humans, cell reprogramming, and mammalian brain development. Ryan returned to Perth in 2012 to establish his own research group, and is currently an NHMRC Investigator and HHMI International Research Scholar, and head of the Epigenetics and Genomics laboratory at the Harry Perkins Institute of Medical Research and UWA. Ryan's laboratory is focused upon understanding the role of the epigenome in regulating cell identity, and developing molecular tools to manipulate the epigenome and gene activity to control cell functions.

Molecular dynamics of human brain development

Oz Single Cell

Abstract Selected Oral

Benchmarking recently available alternative single cell/nuclei profiling kits

Irina Kuznetsova [1], Matthew E Jones [1], Caleb Thomas [1], Khaing Hmon [1] ,Gina Ravenscroft [1], Sue Fletcher [2], Elena Denisenko [1], Alistair R R Forrest [1]

[1] Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, The University of Western Australia, Nedlands, Perth, WA 6009, Australia
[2] Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University WA 6150 and PYC Therapeutics, Nedlands, WA 6009

The vast majority of single cell profiling worldwide has to date used the highly successful 10x genomics microfluidic kits and controllers. Although robust and allowing rapid uptake of single cell profiling, the costs of experiments are still a barrier to entry for many labs and limit our ability to carry out studies on clinical samples of sufficient size and power. Recently, multiple new alternative single cell profiling technologies have become commercially available that promise to substantially reduce the cost per sample. Here, we share our efforts evaluating several of these kits and multiplexing approaches: 10x 3' v3.2 (unpooled, cellplex pooling, genetic pooling), 10x fixed RNA assay, Parse Bio, HIVE, Singleron, and Scipio. We will present our systematic bioinformatic analyses of the data including comparisons of cell composition, genes, and UMIs observed when these technologies are applied to samples of peripheral blood mononuclear cells (PBMCs) and single nuclei isolated from frozen skeletal muscle biopsies, from multiple donors.

Technology Panel

Debuting this year is our technology panel, lead by Associate **Professor Luciano Martelotto**

Discussion points:

- Where is single-cell sequencing going?
- Multi-dimensions vs. multi-omics
- Reproducibility and progress in a single-cell world

Featuring:

A/ Prof Jess Mar

Dr Heather Lee

Professor Alistair Forrest

Dr Oliver Braubach

An engaging and stimulating way to challenge your own impressions and form new ideas on where single cell technologies may lead us.

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Poster Session I

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Temporal tracking of microglial and monocyte single-cell transcriptomics in lethal flavivirus infection

Alanna G. Spiteri[1-4], Claire L. Wishart[1-4], Duan Ni[2,4,5], Barney Viengkhou[4,8], Laurence Macia[2,4,5], Iain L. Campbell[4,8], Markus J. Hofer[4,6,8], Nicholas J.C. King[1-7]

[1]*Viral Immunopathology Laboratory, Infection, Immunity and Inflammation Research Theme, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Australia*
[2]*Sydney Cytometry, The University of Sydney and Centenary Institute, Sydney, NSW 2006 Australia*
[3]*Ramaciotti Facility for Human Systems Biology, The University of Sydney and Centenary Institute, Sydney, NSW 2006 Australia*
[4]*Charles Perkins Centre, The University of Sydney, Australia*
[5]*Chronic Diseases Research Theme, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Australia*
[6]*The University of Sydney Institute for Infectious Diseases, The University of Sydney, Sydney, NSW 2006 Australia*
[7]*The University of Sydney Nano Institute, the University of Sydney, NSW 2006, Australia*
[8]*School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006 Australia*

The resident parenchymal myeloid population in the central nervous system (CNS), microglia, are strategically positioned to respond to neurotrophic CNS virus evasion. Microglia have been implicated in promoting both disease resolution and progression in the acute and post-infectious phase of virus encephalitis. Viral infection of the CNS results in the substantial recruitment of peripheral immune cells into the brain, with nitric-oxide-producing Ly6Chi inflammatory monocyte-derived cells (MCs) being the primary infiltrate in a mouse model of West Nile virus encephalitis (WNE). In this model inflammatory MCs enhance immunopathology and mortality. However, the contribution of microglia to this response is currently undefined. Here we used a combination of experimental tools, including single-cell RNA sequencing (scRNA-seq), microglia and MC depletion reagents, high-dimensional spectral cytometry and computational algorithms to dissect the differential contribution of microglia and MCs to the anti-viral immune response in severe neuroinflammation seen in WNE. Analysis of scRNA-seq data identified 6 unique microglia and 3 unique MC clusters that were predominantly timepoint specific, demonstrating substantial transcriptional adaptation with disease progression over the course of WNE. While microglia and MC adopted unique gene expression profiles, gene ontology enrichment analysis, coupled with microglia and MC depletion studies, demonstrated a role for both of these cells in the trafficking of peripheral immune cells into the CNS, T cell activation and viral clearance. Over the course of infection microglia transitioned from a homeostatic, to an anti-viral and then into an immune-cell recruiting population. Conversely, MC adopted antigen-presenting, immune-cell recruiting and Nos-producing phenotypes, which all had anti-viral function. Overall, this study, for the first time defines the transcriptomic responses of microglia and MCs over the course of WNE, demonstrating both protective and pathological roles of these cells that could potentially be targeted for differential therapeutic intervention to dampen immune-mediated pathology, while maintaining viral clearance functions.

Spatio-temporal landscape of cellular interaction and immune cell dynamics in traumatic brain injury

Duy Pham[1], Emily F. Willis[2], Marc J. Ruitenberg[2], Jana Vukovic[2], Quan H. Nguyen[1]

[1]*Institute for Molecular Bioscience, The University of Queensland.*
[2]*School of Biomedical Sciences, Faculty of Medicine , The University of Queensland*

Following traumatic brain injury (TBI), the neuroinflammation may cause acute secondary injury which leads to neuropsychiatric problems and neurodegenerative pathologies. Through single-cell transcriptomics, target cell types and pathways of immune response to TBI have been explored in recent years. However, global transcriptional changes reflecting cellular responses through cell-cell interactions remain elusive in physical space and time. In this study, we performed the spatio-temporal analysis using Spatial Transcriptomics to understand the immune response across sectioned TBI tissues in different conditions and time points, including transgenic mice with microglia depletion. We confirmed the microglia cells, the resident immune cells of the brain, rapidly change states and communicate with other cell types during the response to their spatial environment. We found in samples collected post-TBI at 6 hours and 3 days that microglia were distributed at a remarkably higher density at hippocampal regions and injured sites compared to in the naive brain and sham control. In the post-TBI 3 days sample, we found a significant cellular signaling event mediated by astrocytic colony-stimulating factor-1 (CSF1) and microglial CSF1 receptor (CSF1R) as the target ligand-receptor pair between astrocyte and microglia. This interaction change was specific in the hippocampal region. Furthermore, our data suggest significant changes in the distribution of other immune, glial, and neuron cell types, contributing to the overall spatial heterogeneity of responses to TBI. Our spatial transcriptomics analysis of immune responses in TBI brain samples across space and time confirmed and added new insights into the pathogenic pathways in TBI and related neuroinflammation disorders.

Single-cell RNA sequencing reveals how conventional dendritic cell heterogeneity is affected during systemic inflammation

Cindy Audiger[1], Tom Weber[1], Sara Tomei[1] and Shalin H. Naik[1]

[1]Walter and Eliza Hall Institute of Medical Research

Dendritic cells (DCs) patrol the organism to detect danger signals such as pathogens or cancer. On activation, DCs migrate to lymphoid organs to activate T cells that will then clear the pathogen or cancer. However, DCs are highly heterogeneous and consist in different subsets with distinct function and abilities to initiate an immune response. It is of major interest to understand how DC heterogeneity is affected by systemic TLR-activation to ensure their maximal efficacy in controlling tumour growth in an immunotherapeutic setting.

To fully understand how DC heterogeneity is affected during systemic inflammation following treatment with a TLR-agonist, we undertook transcriptomic profiling at the single cell level with or without stimulation with Poly I:C in vivo, a TLR-3 agonist used in the clinic.

We identified 30 clusters with distinct transcriptomic signatures. 10 clusters were consistent with a migratory DC signature (CCR7) with 5 of them increasing following Poly I:C treatment. These subsets differed between each other in their expression of IFN-inducible genes, chemokines and apoptotic related transcripts. Among the cDC1 clusters, Poly I:C treatment increased one subset of cells expressing high levels of Cxcl9 transcript, a chemokine involves in T cells recruitment. Interestingly, despite not expressing TLR-3, poly I:C treatment increased numbers of cells in two cDC2 clusters with high expression of IFN inducible genes. This revealed that a bystander effect is sufficient to allow cDC2 maturation. Lastly, systemic Poly I:C inflammation differentially affected splenic and lymph node DCs with clusters specific to each.

Overall, this study reveals the heterogeneity of DCs present at steady state and how they are affected by systemic inflammation. Moreover, the different populations of DCs induced after poly I:C treatment present with distinct transcriptomic phenotypes suggesting different functions and thus roles in initiating an adaptative immune response, which will be the subject of future investigations.

Defining lymphocyte differentiation in the spleen during malaria with spatial transcriptomics at near single-cell resolution.

Cameron G. Williams[1], Evan Murray[2], Hyun Jae Lee[1], Megan S. F. Soon[3], Jessica A. Engel[3], Fei Chen[2], Ashraful Haque[1]

[1]Department of Microbiology and Immunology, University of Melbourne at The Peter Doherty Institute for Infection and Immunology, Parkville, VIC, Australia
[1]Broad Institute of MIT and Harvard, Cambridge, MA, USA
[3]QIMR Berghofer Medical Research Institute, Herston, QLD, Australia

Malaria is an infectious disease that exerts a major toll on human health, with resistance emerging to first-line antimalarial drugs, and few available options for effective vaccination. Improved understanding of cellular immunity to blood-stage malaria parasites may offer strategies for boosting protection in humans. Adaptive immune responses to malaria are dominated by two subsets of CD4+ T helper cells in the spleen: T helper 1 (Th1) cells, which produce inflammatory cytokines, and T follicular helper (Tfh) cells, which interact with B cells to facilitate high-affinity, parasite-specific antibodies. Our previous reports suggested other immune cells interact with differentiating parasite-specific CD4+ T cells to support Th1/Tfh fates. However, direct evidence of these cell-cell interactions remains lacking. To understand CD4+ T cell differentiation within the tissue context of the spleen, we performed parallel scRNA-seq and spatial transcriptomics at 10 micron resolution with Slide-seqV2, using spleen samples from mice infected with blood-stage, rodent-infective Plasmodium chabaudi chabaudi AS. By integrating single-cell and spatial transcriptomic data using Robust Cell-Type Decomposition (RCTD) and cell2location, we first confirmed our capacity to discern microanatomical immune and stromal cell structures in the spleen, as well as change upon infection. Next, Tfh-like transcriptomes were confirmed to localize to B cell zones. Th1 cells co-located with monocytes and cDC2s in red pulp and marginal zones, consistent with roles for those cells supporting Th1 fates. Unbiased assessments of cell-cell co-locations for ~20 different cell types and states suggested novel interactions that are currently being investigated in silico, for example with CellChat, and in vivo with microscopy and cell depletion. Ongoing efforts include mapping single cells in space using CellTrek to achieve true cellular resolution. Thus, 10 micron-resolved spatial transcriptomics at genome-scale facilitated assessment of CD4+ T cell differentiation during malaria and revealed novel cell-cell interactions that might influence malaria immunity.

Use spatial transcriptomics to study tumor predictors in patients with early invasive melanoma

Chenhao Zhou[1], Samuel X Tan[1], Yung-Ching Kao[1], Magdalena Claeson[3], Susan Brown[1], Duncan Lambie[4], David C Whiteman[3], H Peter Soyer[1], Mitchell S. Stark[1], Quan Nguyen[2], Kiarash Khosrotehrani[1]

[1]The University of Queensland Diamantina Institute, Translational Research Institute, Woolloongabba, Queensland 4102, Australia
[2]Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland 4072, Australia
[3]QIMR Berghofer Medical Research Institute, 300 Herston Road, Herston, QLD 4006, Australia. [4]IQ Pathology, Brisbane, Australia; Pathology Queensland, Princess Alexandra Hospital, Brisbane, Australia

Early invasive melanomas, despite their excellent prognosis, account for the majority of melanoma deaths. There is currently a lack of accurate clinical and pathological predictors to identify which patients, with early invasive melanomas, are at the highest risk of disease progression. The recent advances in spatial transcriptomic technologies allow unbiased discovery of key gene and spatial biomarkers controlling the fate of tumorigenesis. Using the 10x Genomics Visium platform, we examined spatially resolved transcriptome profiles of archived FFPE tissues derived from 40 locally invasive thin (<1mm) melanoma patients (20 pairs of fatal cases and nonfatal controls matched for age, sex, year of diagnosis, length of follow-up and tumour thickness) in a well-established and annotated QLD Cancer Registry population cohort. We identified a list of differentially expressed genes associated with patient survival. In particular, case tumours show increased expression of EMT and TNFα signalling-associated genes and decreased expression of antigen processing and presentation (APP)-related genes when compared to control tumours. Interestingly, EMThi tumours and APPhi tumours were located in close proximity to fibroblast and myeloid cell infiltrate respectively, implying that different cross-talks between tumour cells and non-tumour cells could be one major contributor to modulate tumour microenvironment and determine the fate of tumour progression. Further analysis will focus on integrating multiple candidate features and validating them to identify best predictive scenarios for patient survival that can progress into the clinic.

Connecting transcriptomes across neuroinflammatory disease reveals conserved and unique microglial and monocyte response programs

Claire L. Wishart[1-4], Alanna G. Spiteri[1-4], Giuseppe Locatelli[6], Nicholas J.C. King[1-5]

[1]Infection, Immunity, Inflammation Research Theme, Faculty of Medicine and Health, School of Medical Sciences, The University of Sydney, Australia
[2]Sydney Cytometry Facility, The University of Sydney and Centenary Institute, Australia [3]Ramaciotti Facility for Human Systems Biology, The University of Sydney and Centenary Institute, Australia
[4]Charles Perkins Centre, The University of Sydney, Australia
[5]Marie Bashir Institute for Infectious Diseases and Biosecurity (MBI), Sydney Medical School, The University of Sydney, Australia
[6]Theodor Kocher Institute, University of Bern, Bern, Switzerland

Microglia and bone marrow-derived monocytes are key elements of central nervous system (CNS) inflammation, both capable of enhancing and dampening immune-mediated pathology, as highlighted by their phenotypic and functional heterogeneity across disease. However, the study-specific focus on individual cell types, disease models or experimental approaches has compartmentalized our knowledge, limiting our ability to infer common and disease-specific responses. We have integrated single-cell transcriptomic data sets from multiple disease models of autoimmunity, neurodegeneration, and traumatic injury to build a comprehensive resource connecting myeloid responses across six models of neuroinflammation. Transcriptional analysis identified a core set of functionally-defined myeloid states that are conserved across each disease model. These core states included inflammatory, neurodegeneration-associated, interferon-responsive, phagocytic, antigen-presenting, and lipopolysaccharide-responsive cellular states. We then used single-cell RNA-sequencing to evaluate the presence of these myeloid states in CNS viral infection. This revealed the specialized expansion of inflammatory, interferon-responsive and antigen-presenting myeloid states in response to infectious stimuli, showcasing the highly specialised nature of these core myeloid states. Together, this work establishes a unified perspective of myeloid behaviour across CNS pathologies by dissecting the influence of disease environment on shared immune response pathways.

Analysis of population genetic and phenotypic diversity reveal a predictable organisation of gene programs in the cell

Dalia Mizikovsky [1], Marina Naval Sanchez [1], Christian M. Nefzger [1], Gabriel Cuellar Partida [2], Nathan J. Palpant [1]

[1] *Institute for Molecular Bioscience, Brisbane, Australia*
[2] *The University of Queensland*

Background**:** Genome wide association studies (GWAS) define how genetic variation influences phenotypic variation by providing gene-trait associations. We hypothesised that genes with shared functions influence complex trait phenotypes in similar ways. Therefore, sampling a sufficiently diverse landscape of gene-trait variation provides an unsupervised strategy to parse the organisation of cellular gene programs.

Methods. MultiXcan analysis was performed for 1393 complex traits sampled from ~400,000 individuals to generate a gene-trait association matrix in which each gene is linked with each phenotype by the significance of their association. Genes were clustered using dimensionality reduction methods from Seurat and consolidated into a consensus matrix for hierarchical clustering.

Results: 16,849 genes were clustered into 242 unique gene groups predicted to share biological functions based on their pattern of association with complex traits. Gene clusters were significantly enriched for known biological gene sets and protein-protein interactions governing development, signalling, disease, and homeostasis, with exquisite specificity for the top ranked gene-ontologies across all clusters. We show that genes associated with an independent GWAS phenotype reproducibly cluster within our identified gene modules, indicating that genetic effects on complex trait phenotypes are biologically conserved and predictable. Furthermore, our clustering predictions can identify gene programs influencing complex traits from underpowered and transethnic GWAS without requiring increased cohort sizes to increase statistical power. Lastly, we show that despite the considerable size of the UK Biobank, the data does not saturate gene clustering predictions and therefore will improve in quality and accuracy as more data become available.

Conclusion**:** Our analysis provides a novel approach for predicting genetic mechanisms of development and disease by identifying genes coordinating cell function in an unsupervised way using large-scale GWAS data. As such, we demonstrate that the effect of genetic variation on biological phenotypes is predictable.

Deciphering the non-genetic determinants of malignant clonal dominance and therapeutic resistance

Dane Vassiliadis[1,2], Katie A. Fennell[1,2], Enid Y. N. Lam[1,2], Laure Talaramain[1,2], Mark A. Dawson[1,2,3]

[1]Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
[2]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Victoria, Australia
[3]The University of Melbourne Centre For Cancer Research, The University of Melbourne, Melbourne, Victoria, Australia

Intra-tumoral heterogeneity is a driving force of tumour evolution that partitions cancers into a functionally diversified collection of malignant subclones. Whilst the genetic properties (i.e. mutations) that impact malignant cell fitness have been thoroughly investigated, understanding the non-genetic properties (i.e. transcriptional or epigenetic heterogeneity) that contribute to tumour evolution requires a new set of tools that link the initial cellular state to the downstream cellular fate. To this end we developed Single-cell Profiling and LINeage TRacing (SPLINTR), a synthetic lentiviral barcoding strategy that binds the lineage identity of a cell to its corresponding cellular transcriptome, allowing the pre-existing and acquired malignant cell states that impact disease outcome to be identified. Applying SPLINTR to a clinically relevant mouse model of acute myeloid leukaemia (AML), we find that leukaemic stem cells (LSC), malignant cells that drive tumour progression, display heritable clone-intrinsic properties of high, and low clonal output that contribute to the overall tumour mass. Moreover, we show that clonal output is a fundamental principle governing disease invasion into extramedullary sites outside the bone marrow niche, whereby high and low output clones claim mutually exclusive spatial territories that possess distinct transcriptional profiles. Finally, we show that while high and low output LSC clones adapt differently to potent chemotherapy, they co-ordinately emerge from minimal residual disease with increased expression of the LSC program to drive AML recurrence. Together these data provide fundamental knowledge into the non-genetic processes that underpin malignant clonal fitness and therapeutic response with insights that will inform future treatment strategies.

Demuxafy: Improvement in droplet assignment by integrating multiple single-cell demultiplexing and doublet detection methods

Drew Neavin[1], Anne Senabouth[1], Jimmy Tsz Hang Lee[2], Aida Ripoll[3], Himanshi Arora[1] ,sc-eQTLGen Consortium, Lude Franke[4], Shyam Prabhakar[5], Chun Jimmie Ye[6-9], Davis J. McCarthy[10-11], Marta Melé[3,10] , Martin Hemberg[2,12], Joseph E. Powell[1,13]

[1]Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute for Medical Research, Darlinghurst, NSW, Australia
[2]Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK
[3]Life Sciences Department, Barcelona Supercomputing Center, Barcelona, Catalonia, Spain
[4]Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
*[5]Systems Biology and Data Analytics, Genome Institute of Singapore, A*STAR, 60 Biopolis Street, Singapore*
[6]Bakar Institute for Computational Health Sciences, University of California, San Francisco, San Francisco, CA, USA
[7]Institute for Human Genetics, University of California, San Francisco, San Francisco, CA, USA [8]Division of Rheumatology, Department of Medicine, University of California, San Francisco, San Francisco, CA, USA
[9]Chan Zuckerberg Biohub, San Francisco, CA, USA
[10]Bioinformatics and Cellular Genomics, St Vincent's Institute of Medical Research, Fitzroy, Australia
[11]Melbourne Integrative Genomics, School of BioSciences–School of Mathematics & Statistics, Faculty of Science, University of Melbourne, Melbourne, Australia
[12]Present address: Evergrande Center for Immunologic Disease, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA
[13]UNSW Cellular Genomics Futures Institute, University of New South Wales, Kensington, NSW, Australia

With the ability to assay thousands of cells in a single-cell capture, pooling samples from multiple individuals has become a common strategy. This has fueled recent ground-breaking work that merges population genetics with cellular genomics. These studies are built on the ability to assign droplets to a specific individual by using their inherent genetic differences, and multiple computational methods have been developed to demultiplex pools using genetic data. Another challenge implicit with droplet-based single-cell methods is the occurrence of doublets. The inaccurate assignment of cells to individuals or failure to remove doublets contribute unwanted noise to the data and result in erroneous scientific conclusions. Therefore, it is essential to assign cells to individuals and remove doublets accurately.

We present a new framework to improve individual singlet classification and doublet removal through a multi-method intersectional approach. The accuracy was assessed using scRNA-seq data of ~1.4 million peripheral blood mononucleated cells from 1,034 unrelated individuals and ~90,000 fibroblast cells from 81 unrelated individuals. We show that our approach significantly improves droplet assignment by separating singlets from doublets and classifying the correct individual compared to any single method. We show that the best combination of techniques varies under different biological and experimental conditions, and we present a framework to optimise cell assignment for a given experiment. We offer Demuxafy - a framework built in Singularity to provide clear, consistent documentation of each method and additional tools to simplify and improve demultiplexing and doublet removal. Our results indicate that leveraging multiple demultiplexing and doublet detecting methods improves accuracy and, consequently, downstream analyses in multiplexed scRNA-seq experiments.

Convalescent individuals with previous severe COVID-19 have increased abundance of interferon-expressing neutrophils in circulation

Dylan Sheerin[1,2], George Ashdown[1,2], Rachel Evans[1], Kha Phan[1,3], James Cooney[1], Elizabeth Sergison[4], Lauren Wasson[4], Irene Whitney[4], COVID Profile Consortium[1], Emily M Eriksson[1,2], Anna K Coussens[1,2,5]

[1] Walter and Eliza Hall Institute of Medical Research, VIC 3052, Australia
[2]The University of Melbourne, Department of Medical Biology, VIC 3052, Australia [1]
[3]La Trobe Institute for Molecular Science, Department of Biochemistry and Chemistry, School of Agriculture, Biomedicine and Environment, La Trobe University, Melbourne, Australia
[4]Honeycomb Biotechnologies Inc., Waltham, Massachusetts, United States of America
[5]Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine, Department of Pathology, University of Cape Town, South Africa

Neutrophils have classically been considered short-lived, transcriptionally inactive, terminally differentiated cells; however, there is growing appreciation for the heterogeneity of this cell type and their gene expression changes during infection. Neutrophils have been implicated in COVID-19 pathogenesis, particularly through the dysregulated release of extracellular traps (NETs) which have been shown to be elevated in hospitalised patients.

To determine the transcriptional programmes which underlie neutrophil-related pathogenesis and whether a phenotype likely to exhibit dysregulated responses could be identified, we performed single-cell RNA-sequencing (scRNA-seq) on neutrophils isolated from individuals who had recovered from COVID-19. Neutrophils were isolated by gradient centrifugation from whole blood samples taken from age- and sex-matched individuals who experienced severe (n = 4), mild/moderate (n = 4) or no symptoms (n = 4). A total of 15,000 cells per donor were captured for scRNA-seq. The remaining neutrophils were infected with SARS-CoV-2 at an MOI of 3 and the same number of cells were captured for scRNA-seq at 1-, 2- and 3-hours post-infection in addition to NETosis quantification by live-cell confocal microscopy.

Clustering analysis was performed across all 48 samples (12 participants, 4 time-points) and grouped neutrophils according to positive expression markers. Comparing the proportions of each cluster between the three disease severity groups at each time point revealed a greater abundance of neutrophils expressing a core set of IFN-related genes, including IFIT2, IFIT3, IRF1, and STAT1, in the severe COVID-19 group at the pre-infection time point. The proportion of this cluster increased over the 3-hours of SARS-CoV-2 infection in the severe and mild/moderate groups, but remained consistently low in the asymptomatic group.

This analysis revealed transcriptional divergence in steady-state neutrophils between individuals with distinct COVID-19 infection outcomes. This divergence persisted over the course of ex vivo re-infection with SARS-CoV-2, indicating that these IFN-expressing neutrophils may represent a prognostic phenotype and influence disease outcome.

A toolbox for analysing single-cell proteomics data from LEGENDScreen assay

Hsiao-Chi Liao[1,2,3], Terence P. Speed[1,2], Davis J. McCarthy[1,3,5], Agus Salim* [1,2,4,6,7]

[1]*School of Mathematics and Statistics, University of Melbourne, VIC 3010, Australia*
[2]*Bioinformatics Division, Walter and Eliza Hall Institute of Medical Research, VIC 3052, Australia* [3]*Bioinformatics and Cellular Genomics, St Vincent's Institute of Medical Research, VIC 3065, Australia*
[4]*Melbourne School of Population and Global Health, University of Melbourne, VIC 3053, Australia*
[5]*Melbourne Integrative Genomics, Faculty of Science, University of Melbourne, VIC 3010, Australia*
[6]*Baker Heart and Diabetes Institute Melbourne, VIC 3004, Australia*
[7]*Department of Mathematics and Statistics, La Trobe University, VIC 3086, Australia*

The LEGENDScreen assay allows cost-effective quantification of more than 200 surface proteins at single-cell resolution. The Infinity Flow protocol (Becht et al., 2021) was developed and used to measure highly informative protein markers (termed 'backbone') on all cells in wells distributed across three 96-well plates, and well-specific exploratory protein markers (termed 'infinity'), followed by using the backbone markers to impute the infinity markers at all wells using machine learning methods. This protocol offers unprecedented opportunities for more comprehensive classification of cell types. However, several aspects can be improved, including methods for background correction and removal of unwanted variation. We propose an end-to-end toolbox that carefully pre-processes the raw data in FCS format, and further imputes the 'missing' infinity markers in the wells without measurement. Our pipeline starts by performing background correction on raw intensities to remove the noise from electronic baseline restoration and fluorescence compensation by adapting the normal-exponential convolution model. Secondly unwanted technical variation such as batch effects is removed by applying the log-normal model with plate, column, and row factors. Thirdly, imputation is done by using the informative backbone markers as predictors. Lastly, cluster analysis and other statistical analyses can be performed on the completed dataset. Unique features of our approach relative to existing methods include doing background correction prior to imputation and removing unwanted variation from data at the cell-level while explicitly accounting for the potential association between biology and unwanted factors. We benchmark our pipelines against alternative pipelines and demonstrate that our approach is better at preserving biological signals, removing unwanted variations, and imputing unmeasured infinity markers.

A Genome-wide Epigenetic Repressive Signature Reveals Genetic Regulators of Cell Identity at Single Base Resolution

Enakshi Sinniah[1], Sophie Shen[1], Dalia Mizikovsky[1], Woo-Jun Shim[1], Zhili Zeng[1], Jian Zeng[1], Sonia Shah[1], Mikael Boden[1], Nathan J. Palpant[1]

[1]Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia
[2]School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia

Understanding genome-wide regulation of cell decisions and functions is essential for establishing mechanisms of development, disease, and complex traits. Yet, causal inference remains challenging as >90% of disease-associated genetic variants map to non-coding regions of the human genome, and current methods for variant prioritization are often limited to annotated elements like protein-coding genes. Here, we analyzed 833 epigenomes from the EpiMap repository to identify distinguishing features of genome regulation associated with cell-type specification. We show that the a priori deposition of genome-wide H3K27me3 across diverse cell-types, which we call a repressive tendency score (RTS), provides an approach to enrich for coding and non-coding features of the genome governing cell identity. While only 1.4% of the human genome have significant RTS values, these prioritized regions are enriched in genes, non-coding RNAs, cis-regulatory elements and SNPs associated with fundamental regulation of cell differentiation, organ morphogenesis and disease. We tested the ability to prioritize causal genetic variants contributing to complex traits and diseases by interfacing genome-wide RTS values with data on human genetic variation. Analysing millions of genetic variants from databases including the UK Biobank and ClinVar, we offer an unsupervised approach to rank variant pathogenicity in a disease-agnostic manner to prioritize causal variants, particularly contributors to congenital disorders. Further, we use the RTS metric to systematically predict >8000 cell-type specific cis-regulatory elements, including novel enhancers, silencers, and bifunctional regulatory elements to expand our understanding of regulatory control of cell-type specification. We validate the predictive power of our computational method by analysing CAGE-seq data to identify and experimentally validate a lncRNA, RP11-175E9.1, as a novel regulator of hiPSC differentiation in vitro. Together, this study demonstrates that the conservation of epigenetic regulatory logic provides an effective strategy for studying any omics data-type to infer cell-type specific mechanisms that underpin molecular regulation of cell identity.

Nuclei Segmentation Techniques for Improving STOmics Analyses

Harrison A Mueller[1,2], Xiaohuan Sun[3], Chunhua Li[3], Qiqi He[3], Amitava Datta[2], Max Ward[2], Alistair R R Forrest[1], Elena Denisenko[1]

[1]Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, The University of Western Australia, Nedlands, Perth, WA 6009, Australia
[2]Department of Computer Science and Software Engineering, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia
[3]BGI-Shenzhen, Shenzhen 518103, China

Spatial transcriptomics is becoming an increasingly important technique for cellular biology and has led to numerous insights in neuroscience, developmental biology, and cancer research. Several tools have been developed to measure gene expression as well as the spatial origin of a given transcript, with one of the latest being STOmics. Based on Next-Generation Sequencing, STOmics has a subcellular resolution, much greater than previous NGS-based tools. However, this subcellular resolution has some drawbacks, such as spots no longer representing cells or nuclei.

One way to solve this is to use AI and machine learning techniques to segment nuclei based on single-stranded DNA stains of the sequenced tissue section. By applying these maps to the STOmics data, we can attain per nuclei counts.

Here we compare a couple of published tools, Cellpose and DeepCell, to conventional segmentation techniques and a neural network trained specifically for this task. To compare and contrast these tools, we have compared the segmentation maps with pre-mRNA counts, which align to nuclei; and we have investigated the accuracy of these tools on computer-generated ssDNA stain images. Finally, we compared down-stream analyses to determine the overall effect of segmentation on the results.

Leveraging single-cell RNA-sequencing atlases to understand gene essentiality

Huiwen Zheng [1], Atefeh Taherian Fard [1] ,Ernst Wolvetang [1] , Jessica Cara Mar [1]

[1] *Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia*

Gene essentiality emerged as a concept associated with the notion of the minimal genome, with a gene considered essential when the loss of its function compromises an organism's viability. Advances in gene editing techniques like CRISPR-Cas9 have provided insight into the core set of essential genes. However, considerable discrepancies exist between essential gene sets identified using in vitro human cell lines and in vivo mouse models, demonstrating the need for a more comprehensive investigation to understand gene essentiality across both species and cell types. Recently, single-cell RNA-sequencing (scRNA-seq) atlases have provided an opportunity to explore gene expression under normal conditions in a cell-type and organism-specific manner. Here, we use the Tabula-Muris and Tabula-Sapian atlases to examine the transcriptomic profiles of in vivo mouse and human models to identify common essential genes across cell types. Using datasets developed for benchmarking sequencing methods, we further refine these essential genes to those that are not only biologically critical across a large number of cell types but also robustly detectable at single-cell levels, denoted as scEssentials. scEssentials identified for both the mouse and human models were consistently highly-expressed across more than 60 cell types. We demonstrate a large number of significantly correlated gene pairs within scEssentials, which produce densely connected coexpression networks. Finally, we develop a score to quantify the relative importance of genes within scEssentials, which is found to correlate inversely with the gene mutation frequency index. Furthermore, only one-fifth of scEssentials showed ageing-related differential-expression when investigated using the Tabular-Muris-Senis, with dysregulation occurring primarily in stem cells of varying tissue origins. Collectively, the stability of scEssentials serves as a reference for analysing scRNA-seq data, and provides insight into the heterogeneous nature of biological processes such as ageing.

Single-cell RNAseq of adult mouse brain and meningeal vasculature

Lucas Tobar [1,2], Oliver Cucanic [1,2], Niko Thio [1,2], Timothy Semple [1,2], Alicia Oshlack [1,2,3,4], Rae Farnsworth [1,2], Steven Stacker [1,2,5]

[1] Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia,
[2] Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, VIC 3010, Australia,
[3] Peter MacCallum Cancer Centre, Computational Biology Program, Parkville, Australia,
[4] School of Mathematics and Statistics, University of Melbourne, Parkville, Australia,
[5] Department of Surgery, Royal Melbourne Hospital, University of Melbourne, Parkville, VIC 3050, Australia

The brain contains highly specialised blood vessels lined by endothelial cells (EC) that are strongly adhered to each other through tight junctions. These ECs are further encapsulated by pericytes, astrocytes and smooth muscle cells (SMC), forming a highly selective barrier to circulating factors known as the blood-brain barrier. Single-cell isolation of mouse brain ECs is thus challenging, requiring extensive enzymatic and mechanical digestion, at the expense of fragile neuronal cells. The meninges are membranes surrounding the brain, containing unique vasculature and fluid drainage networks. Meningeal cells are extracted from the meningeal tissue adhered to the inner surface of the skull, also presenting challenges, due to low cell abundance, and cell fragility.

A scRNAseq analysis was performed on brain and meningeal cells isolated from n=6 individual barcoded (TotalSeq-C) and pooled BALB/c mice. Flow-cytometry sorting was used to obtain viable CD45-/CD11b-/TER119-/CD31+ (endothelial cells), and CD45-/CD11b-/TER119-/CD31- (other stromal cells) fractions, each run on an individual 10X Chromium capture.

After quality control, 10650 brain CD31-, 14421 brain CD31+, 4152 meninges CD31-, and 2701 meninges CD31+ cells were recovered. Cells were annotated using the annotation tool Azimuth and data from published mouse brain single-cell analyses. The brain CD31+ fraction consisted almost entirely of ECs, illustrating successful flow-cytometry sorting. Interestingly, the brain CD31- fraction was highly enriched in choroid plexus epithelial cells, suggesting that the extensive digest protocol also isolated other cells known to be tightly adhered and difficult to extract. The meninges CD31+ fraction was highly enriched in ECs, containing populations seen in other studies, such as VWF+ ECs present in the dural sinuses. The CD31- fraction contained fibroblast-like cells, SMCs, pericytes, and other leptomeningeal cells.

This study elucidates the diverse populations of cells obtained through optimised EC isolation protocols, informing the design of future mouse cancer model experiments investigating vascular microenvironments in brain metastasis.

RUV-III-NB: normalization of single cell RNA-seq data

Agus Salim

MSPGH, The University of Melbourne

Normalization of single cell RNA-seq data remains a challenging task. The performance of different methods can vary greatly between datasets when unwanted factors and biology are associated. Most normalization methods also only remove the effects of unwanted variation for the cell embedding but not from gene-level data typically used for differential expression (DE) analysis to identify marker genes. We propose RUV-III-NB, a method that can be used to remove unwanted variation from both the cell embedding and gene-level counts. Using pseudo-replicates, RUV-III-NB explicitly takes into account potential association with biology when removing unwanted variation. The method can be used for both UMI or read counts and returns adjusted counts that can be used for downstream analyses such as clustering, DE and pseudotime analyses. Using published datasets with different technological platforms, kinds of biology and levels of association between biology and unwanted variation, we show that RUV-III-NB manages to remove library size and batch effects, strengthen biological signals, improve DE analyses, and lead to results exhibiting greater concordance with independent datasets of the same kind. The performance of RUV-III-NB is consistent and is not sensitive to the number of factors assumed to contribute to the unwanted variation.

Identifying gene expression programs associated with transcriptional plasticity in single cell RNA sequencing data

Alejandro Casar [1,2], David Goode [1], Howard Bondell [2]

[1] *Peter MacCallum Cancer Centre,*
[2] *University of Melbourne - School of Mathematics and Statistics*

Transcriptional plasticity is a phenomenon where cells reversibly change molecular states by altering their gene expression. Recent evidence suggests transcriptional plasticity is a key driver of the emergence of drug resistance in cancer, and ultimately, therapeutic failure. However, it remains unclear what specific factors drive transcriptional plasticity, and uncovering how it works could have potential implications on how we approach therapy to maximize its effectiveness and improve patient life expectancy.

With the goal of understanding the mechanisms that allows cells to change from one transcriptional state to another that improves their survivability under different environments, we analyzed and tracked bar-coded single cell RNA seq data taken on different conditions. By tracking clone frequencies in vitro, in vivo, and at different time-points under therapeutic pressure we manage to identify genes that are potential indicators of fitness for each circumstance. We then observe their gene expression distribution among cells and from that set of genes we keep the ones that show some degree of bimodality and cluster those genes that are similarly distributed among the tumor into gene expression programs. Finally, by analyzing how these gene expression programs change between the different experimental scenarios we hope to identify genes associated with the ability to change expression to survive therapy.

So far, the pipeline we are creating has been able to identify gene expression programs associated with known cellular states, specifically cell cycle genes S and G2M. Using these results as a baseline we plan to extend the methodology and identify gene expression programs associated to other cellular states of interest, such as sensitive or resistant to therapy, as well as identifying genes that might indicate the ability of a cell to change from once state to another.

scREMOTE: Using multimodal single cell data to predict regulatory gene relationships and to build a computational cell reprogramming model

Andy Tran [1,2], Pengyi Yang [1, 3], Jean Yang [1,2], John Ormerod [1]

[1] *School of Mathematics and Statistics, University of Sydney*
[2] *Charles Perkins Centre, University of Sydney*
[3] *Children's Medical Research Institute, Westmead, Australia*

Cell reprogramming offers a potential treatment to many diseases, by regenerating specialized somatic cells. Despite decades of research, discovering the transcription factors that promote cell reprogramming has largely been accomplished through trial and error, a time-consuming and costly method. A computational model for cell reprogramming, however, could guide the hypothesis formulation and experimental validation, to efficiently utilize time and resources. Current methods often cannot account for the heterogeneity observed in cell reprogramming, or they only make short-term predictions, without modelling the entire reprogramming process. Here, we present scREMOTE, a novel computational model for cell reprogramming that leverages single cell multiomics data, enabling a more holistic view of the regulatory mechanisms at cellular resolution. This is achieved by first identifying the regulatory potential of each transcription factor and gene to uncover regulatory relationships, then a regression model is built to estimate the effect of transcription factor perturbations. We show that scREMOTE successfully predicts the long-term effect of overexpressing two key transcription factors in hair follicle development by capturing higher-order gene regulations. Together, this demonstrates that integrating the multimodal processes governing gene regulation creates a more accurate model for cell reprogramming with significant potential to accelerate research in regenerative medicine.

Oz Single Cell

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Pitfalls and opportunities for applying PEER factors in single-cell eQTL analyses

Angli Xue[1,2], Seyhan Yazar[1], Drew Neavin[1], Joseph E. Powell[1,3]

[1]Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Sydney, NSW, 2010, Australia
[2]School of Medical Sciences, University of New South Wales, Sydney, NSW, 2052, Australia [3]UNSW Cellular Genomics Futures Institute, University of New South Wales, Sydney, NSW, 2052, Australia

Using latent variables in gene expression data can help correct spurious correlations due to unobserved confounders and increase statistical power for expression Quantitative Trait Loci (eQTL) detection. Probabilistic Estimation of Expression Residuals (PEER) is a widely used statistical method that has been developed to remove unwanted variation and improve eQTL discovery power in bulk RNA-seq analysis. However, its performance has not been largely evaluated in single-cell eQTL data analysis, where it is becoming a commonly used technique. Potential challenges arise due to the structure of single-cell data, including sparsity, skewness, and mean-variance relationship. Here, we show by a series of analyses that this method requires additional quality control and data transformation steps on the pseudo-bulk matrix to obtain valid PEER factors. By using a population-scale single-cell cohort (OneK1K, N = 982), we found that generating PEER factors without further QC or transformation on the pseudo-bulk matrix could result in inferred factors that are highly correlated (Pearson's correlation r = 0.626~0.997). Similar spurious correlations were also found in PEER factors inferred from an independent dataset (induced pluripotent stem cells, N = 31). Optimization of the strategy for generating PEER factors and incorporating the improved PEER factors in the eQTL association model can identify 9.0~23.1% more eQTLs or 1.7%~13.3% more eGenes. Sensitivity analysis showed that the pattern of change between the number of eGenes detected and PEER factors fitted varied significantly for different cell types. In addition, using highly variable genes (e.g., top 2000) to generate PEER factors could achieve similar eGenes discovery power as using all genes but save considerable computational resources (~6.2-fold faster). We provide diagnostic guidelines to improve the robustness and avoid potential pitfalls when generating PEER factors for single-cell eQTL association analyses.

Transcriptional Steps in Megakaryocyte Commitment and Maturation

AnneMarie Welch[1], Carolyn de Graaf[1], Maria Kauppi[1], Christine Biben[1], Samir Tauodi[1], Doug Hilton[1]

[1]Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia

Megakaryocytes are large cells that play an important role in haemostasis through the production of platelets, cell-to-cell communication, and maintenance of the bone marrow niche. In particular, platelets are critical to the clotting process and maintenance of vascular integrity. Despite their importance, in vitro production of megakaryocytes and platelets is limited, and the main supply of platelets still relies on plasma donations.

In the bone marrow, megakaryocytes mature from hematopoietic stem cells (HSCs). Within the HSC compartment, two populations can be distinguished based on their self-renewal capacity: long term hematopoietic stem cells (LT-HSCs) and short-term hematopoietic stem cells (ST-HSCs). In the mouse, there are three proposed pathways for megakaryocyte development: classical, alternative, and myeloid bypass. For both the classical and alternative pathway, megakaryocyte progenitors are differentiated from ST-HSCs through multipotent progenitors, and development from these cells occur in a stepwise manner. Unique to the classical model are more restricted progenitors, the common myeloid progenitor (CMP) as well as megakaryocyte-erythroid progenitor (MEP), which have both been shown to develop into megakaryocyte progenitors. In the myeloid bypass pathway, LT-HSCs directly differentiate into megakaryocyte progenitors.

Using flow cytometry, all cells involved in each megakaryocyte maturation pathway were isolated and sequenced through the 10x single cell sequencing platform. With this in vivo dataset, we will investigate and compare megakaryocyte differentiation. In the future, we plan to compare this dataset with an in vitro dataset which will comprise of high-end stem/multipotent progenitor populations that have been isolated and matured into megakaryocytes in culture. This will provide insight into the path to megakaryopoiesis and inform in vitro systems of megakaryocyte - and ultimately platelet - production.

GeoSpatial-scRNA-seq reveals distinct spatial patterning of the tumor microenvironment in hepatocellular carcinoma

Ashley Soet[1,2] Saurabh Gupta[1,3] Rhea Pai[1,3] Shirley Go[4] Jennifer Currenti[1,3] Nina Tirnitz-Parker[3] Peter Leedman[1] Michael Wallace[4] Louise Winteringham[1] Ankur Sharma[1,3,5]

[1]Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, 6 Verdun Street, Nedlands, Perth, Western Australia 6009, Australia.
[2]The University of Western Australia, Perth, Western Australia 6009, Australia.
[3]Curtin Medical School, Curtin Health Innovation Research Institute (CHIRI), Curtin University, Perth, Western Australia 6102, Australia.
[4]Department of Hepatology, Sir Charles Gairdner Hospital, Perth, Australia
*[5]Institute of Molecular and Cellular Biology (IMCB), Agency for Science, Technology and Research (A*STAR), 138673 Singapore.*

Hepatocellular carcinoma (HCC) is the third deadliest cancer worldwide. HCC tumour evolution involves the accumulation of driver mutations, genomic instability, microenvironmental reprogramming and immune evasion. Recently, our laboratory discovered the presence of fetal-like cells in the HCC tumor microenvironment, termed the Oncofetal ecosystem. Oncofetal reprogramming of the tumor microenvironment (TME) orchestrates an immunosuppressive ecosystem. A fundamental yet unresolved question is how oncofetal cells are localised in different GeoSpatial locations of tumors and the impact of subsequent cell-cell interactions in pathobiology. We hypothesise that vascularisation and hypoxia impact spatial patterning of the Oncofetal ecosystem. To comprehend spatial patterning of the Oncofetal ecosystem in HCC we performed GeoSpatial scRNA-seq from distinct anatomical regions representing 12 paired tissues from 6 HCC tumors. Here we analysed ~30,000 single cells and identified ~30 major cell types representing immune, stromal and tumor cells. Further analysis indicates distinct GeoSpatial patterning of oncofetal cells in HCC. This work has implications in understanding the cellular network of oncofetal cells in HCC and its impact on disease progression and therapy response.

Endogenous retroviruses mediate transcriptional rewiring in response to oncogenic signaling in colorectal cancer

Atma Ivancevic[1], David Simpson[1], Edward Chuong[1]

[1]BioFrontiers Institute, University of Colorado Boulder, CO, USA

Repetitive elements such as endogenous retroviruses shape chromatin landscapes. They facilitate the generation of novel cell-type-specific regulatory networks by replicating themselves and dispersing regulatory sequences throughout the genome. Despite their recognized importance in cell biology, repetitive sequences continue to be understudied, especially at the single cell level. This is particularly concerning for the study of immunity and/or disease, where bulk data do not capture the heterogeneity of these tissues. Recently, we used bulk sequencing data and CRISPR experiments to show that ancient LTR10 elements derived from the HERVI retrovirus can reactivate as tumor-specific enhancers in human colorectal cancer cells. LTR10 enhancers facilitate AP1-dependent oncogenic signaling pathways by regulating genes associated with tumorigenesis, including autophagy factor ATG12 and DNA repair factor XRCC4. We further discovered that individual LTR10 sequences contain variable number tandem repeat regions that affect AP1 binding activity. Our work implicates endogenous retroviruses as a source of pathological regulatory variants that facilitate transcriptional rewiring in cancer. Preliminary evidence shows that LTR10-associated transcripts are overexpressed in single cell RNA-seq from patient tumors. To investigate this more broadly, we are developing a pipeline to detect and predict repeat-derived regulatory networks at the single cell level. Our pipeline, scRepeatNet, will enable single cell analysis of repetitive sequences in three key ways. First, by using open-source packages, such as SnapATAC and GIGGLE, to identify candidate repeat-derived regulatory elements enriched in specific cell subpopulations. Second, by using co-accessibility to link putative enhancers to target gene promoters, defining repeat-derived regulatory networks for each cell subpopulation. Third, by training a model based on transcriptome data to predict repeat-derived regulatory networks in new cell subpopulations. We aim to investigate the full impact of repetitive and retroviral sequences in different cell subpopulations and reveal potential regulatory sequences to therapeutically target in diseases such as cancer.

Building a cellular atlas of localised luminal breast cancers

Beata M. Kiedik[1,2], Daniel Lee Roden[1,2], Kate Harvey[1], Ghamdan Al-Eryani[1,2], Sunny Z. Wu[1,2], Mun N. Hui[1,3], Sandra O'Toole[1,2], Elgene Lim[1,2], Charles M. Perou[4], Alexander Swarbrick[1,2]

[1]The Kinghorn Cancer Centre and Cancer Research Theme, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia
[2]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, NSW 2052, Australia
[3]Chris O'Brien Lifehouse, Camperdown NSW, Australia
[4]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

While the number of deaths from breast cancer is decreasing and the disease-free survival has been significantly prolonged; there is still a large number of patients with ER-positive (ER+) disease that do not respond to the therapy or relapse. Therefore, for many patients the chosen therapy is inadequate and ultimately leads to treatment failure. We hypothesize that this is associated with the cellular heterogeneity of the tumour microenvironment, such as the presence of a heterogeneous mix of neoplastic cells with distinct molecular subtypes.

Single cell technologies have provided powerful tools to address this issue, by allowing for better understanding of breast cancer heterogeneity at an unprecedented level and resolution. Previous studies using single-cell RNA-seq (scRNA-seq) have highlighted remarkable epithelial cell diversity and the presence of different transcriptional patterns across tumours. However, their association with outcome and survival is unclear and focus on the luminal breast cancers, which constitute the majority of breast cancers, is lacking.

This work will comprehensively map the cellular heterogeneity of ER+ breast cancers by generating an integrated cellular atlas of ~200 treatment-naive primary tumours. These samples have been collected over 8 years from breast cancer biopsies and surgeries and have detailed clinical annotation. We have developed cryopreservation and tissue processing workflows to yield high-viability cell suspensions from primary clinical tissue. We have benchmarked and optimized computational processes for data demultiplexing, annotation and integration to permit processing of high-quality single-cell transcriptomes from more than one million cells.

This is the first study of ER+ breast cancers, at this scale, at cellular resolution. We hypothesize it will unravel and define novel cellular subtypes and interactions that drive the adverse events in ER-positive breast cancer patients, leading to high translational impact.

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Detection of significantly different populations of cells in single cell RNA-seq data

Brad Balderson[1], Stefan Thor[2], Mikael Boden[1]

[1]*School of Chemistry and Molecular Biosciences, Brisbane, Australia*
[2]*School of Biomedical Sciences, Brisbane, Australia*

Standard analysis of single cell RNA-seq data includes normalisation of read counts, gene/feature selection, dimensionality reduction (Principal components analysis), nearest-neighbour graph construction, and finally clustering and visualisation based upon the nearest-neighbour graph. The most widely used approach for clustering based upon the nearest-neighbour graph is the Leiden algorithm, which takes a resolution parameter in order to 'cut' the graph into discrete communities of cells. To label communities as particular cell types, generally a post-hoc differential expression is performed, and based upon the resulting marker genes the resolution parameter is altered. Often, since the Leiden algorithm considers the nearest-neighbour graph as the primary representation of the single cell data, clusters need to be manually merged when separate clusters express the same marker genes. This manual process is currently unavoidable since lower resolution parameters will result in merging both distinct & non-distinct clusters, hence motivating a higher resolution parameter and manual merging afterward. While this is not generally a problem when dealing with single cell data containing few cell types, this becomes a massive problem when dealing with tens to hundreds of cell populations, as is commonly observed in neuronal single cell RNA-seq data. To alleviate this problem, we developed neurotools, an scverse compatible python package implementing an expectation maximisation algorithm that automates the described merging process from over-clustered single cell data. The resulting output is significantly different single cell populations, the learnt marker genes which differentiate each population from one another, and per-cell enrichment scores of the population marker genes in each individual cell; thus providing a clear and interpretable down-stream diagnostic to show the distinct gene expression differentiating each cluster.

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Knowledge-based Single-cell Clustering and Annotation

Carissa Chen[1,2], Lijia Yu[1,2,3], Pengyi Yang[1,2,3]

[1]Charles Perkin Centre, University of Sydney, Camperdown, NSW 2006
[2]Computational Systems Biology Group, Children's Medical Research Institute, Westmead, NSW, 2145
[3]School of Mathematics and Statistics, University of Sydney, Camperdown, NSW 2006

Single-cell RNA sequencing has become commonplace to interrogate the intrinsic heterogeneity present across biological systems. The first and foremost inquiry of any single-cell dataset involves characterising the observed single cells. Wherein accurate identification is paramount to meaningful extrapolation of the genetic signatures that dictate cellular behaviour. The burst of single-cell atlases has provided a rich resource for supervised cell classification approaches that learn from reference datasets to query single-cell datasets. Other approaches involve unsupervised clustering followed by manual assignment using known molecular markers. However, the challenge remains to estimate the optimal number of clusters and simultaneously perform cell annotation. We introduce a knowledge-driven single-cell clustering method that leverages the wealth of single-cell reference atlases available to determine the ideal partitioning of clusters based on maximal cell type enrichment. We demonstrate its applicability to annotate small-scale single-cell datasets anticipating the demand as targeted single-cell studies become commonplace.

Elucidating the relationships between markers of cellular senescence in human esMSC undergoing replicative senescence

Cecilia Gomez-Inclan, Ebony Rose Watson, Atefeh Taherian Fard, Hannah Leeson, Julio Aguado-Perez, Giovanni Pietrogrande, Dominique Power, Ernst Wolvetang, Jessica Mar.

Australian Institute for Bioengineering and Nanotechnology

Cellular senescence is one of the main drivers of ageing. It is characterised by an irreversible cell cycle arrest accompanied by the expression of specific genes, the increased activity of enzymes such as β-galactosidase, and the secretion of senescence-associated factors. Single cell transcriptomic analysis of several human cell lineages has revealed remarkable heterogeneity in the senescent phenotype within a cell population, although the biological relevance or impact of this variability remains largely unknown. Furthermore, whether this heterogeneity revealed by transcriptomic approaches replicates at a protein level remains largely unexplored. In this project, Mesenchymal Stromal Stem Cells (MSCs) were induced to replicative senescence. Later, single cell immunofluorescence analyses of commonly used markers of senescence (p21, p16, β-galactosidase; BrdU and nuclear characteristics) were conducted at three different timepoints. MSCs presented substantial heterogeneity within the cell populations and across the different timepoints. Additionally, p21 nuclear content correlated poorly with p16 and β-galactosidase, indicating that this protein is likely an early and poor indicator of senescence in these cells. Finally, p16-positive, β-galactosidase-positive cells showed an increased DNA content, suggesting an arrest at the G2 phase of their cell cycle. Overall, this study provides insights about in-population senescent cells heterogeneity and reveals differential content dynamics among the commonly used markers of senescence in MSCs.

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Matilda: Multi-task learning from single-cell multimodal omics

Chunlei Liu[1]

[1]Children's Medical Research Institute, Parramatta, NSW

Single-cell multimodal omics technologies enable multiple molecular programs to be simultaneously profiled at a global scale in individual cells, creating opportunities to study biological systems at a resolution that was previously inaccessible. However, the analysis of single-cell multimodal omics data is challenging due to the lack of methods that can integrate across multiple data modalities generated from such technologies. Here, we present Matilda, a multi-task learning method for integrative analysis of single-cell multimodal omics data. By leveraging the interrelationship among tasks, Matilda learns to perform data simulation, dimension reduction, cell type classification, and feature selection in a single unified framework. We compare Matilda with other state-of-the-art methods on datasets generated from some of the most popular single-cell multimodal omics technologies. Our results demonstrate the utility of Matilda for addressing multiple key tasks on integrative single-cell multimodal omics data analysis.

A comparison of cell multiplexing reagents for scRNA-seq

Daniel V. Brown [1]., Patrick Grave [1], Casey Antilla [1], Ling Ling [1], Tracey Baldwin [1], Ryan Munnings [2], Christine Biben [3], Tom Weber [2], Peter Hickey [1,3], Amelia Dunstone [1,3], Vanessa Bryant [2], Daniela Zalcenstein [1] and Rory Bowden [1]

[1] Advanced Technology and Biology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
[2] Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
[3] Epigenetics and Development Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia

Combining multiple samples into a single experimental unit such as a 10x Genomics capture has the benefit of higher throughput, reduction of batch effects and reduced costs. Multiple reagents have been developed to enable sample multiplexing through different mechanisms. We compared three such reagents; Total-Seq A hashtag antibody, MULTI-Seq lignoceric modified oligo and Cellplex lipid modified oligo.

We first used PBMCs, a cell type robust to ex vivo manipulation. As the PBMCs were from distinct human donors, SNP genotypes were used as ground truth. In a follow-up experiment we compared reagents in a more fragile cell type, mouse embryonic day E18.5 brain. Signal to noise and overall classification accuracy were used as the primary outcomes. Compared to previously described studies we performed more upfront wet-lab optimisation and more detailed dry-lab analysis.

In PBMCs MULTI-Seq was the superior reagent while in mouse E18.5 brain CellPlex performed the best. The extended sample handing required for the fragile cell type comprised the performance of the gene expression assay. To further assess cost saving measures we evaluated the Jumpcode CRISPRclean housekeeping depletion kit, a reagent that depletes ribosomal and mitochondrial genes. We observed a reduction of sequencing requirements in line with the manufacture's claims. No different in cell annotation was observed when housekeeping genes are depleted.

Overall our study provides guidelines and a cost benefit analysis for the usage of cell multiplexing reagents in single-cell RNA-Seq.

Oz Single Cell

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Mutational Profiling of Single Pancreatic Cancer Circulating Tumour Cells

Dannel Yeo [1,2,3,4], Althea Bastian [1,2,3], Heidi Strauss [1,2,3], Aimei Lee [4], Chirag Parsania [2], Payal Saxena [3,5], Charbel Sandroussi [3,6], Sara Wahlroos [7], Peter Grimison [3,7], John EJ Rasko [1,2,3,4]

[1] Li Ka Shing Cell & Gene Therapy Program, The University of Sydney, Camperdown, NSW, 2050, Australia;
[2] Gene & Stem Cell Therapy Program Centenary Institute, The University of Sydney, Camperdown, NSW, 2050, Australia;
[3] Faculty of Medicine & Health, The University of Sydney, Sydney, NSW, 2006, Australia;
[4] Cell & Molecular Therapies, Royal Prince Alfred Hospital, Camperdown, NSW, 2050, Australia;
[5] Division of Gastroenterology, Department of Medicine, Royal Prince Alfred Hospital, Camperdown, NSW, 2050, Australia;
[6] Department of Upper Gastrointestinal Surgery, Royal Prince Alfred Hospital, Camperdown, New South Wales, 2050, Australia;
[7] Department of Medical Oncology, Chris O'Brien Lifehouse, Camperdown, NSW, 2050, Australia

Background: Pancreatic cancer is an aggressive cancer characterised by poor prognosis with a 5-year survival rate of less than 11%. There are limited treatment options available with most patients not responding or progressing rapidly. Sampling tissue involves an invasive biopsy procedure. Circulating tumour cells (CTCs) disseminate via the vascular system and presents a potential non-invasive sampling method. However, an accurate and reliable method to detect these rare cells remains a challenge. Using cell loss-less density-based enrichment of mononucleated blood cells (RareCyte technology), individual CTCs can be detected by fluorescent staining, picked using an on-board cell-picker and characterised. This study aimed to detect, isolate and genetically profile CTCs from pancreatic cancer patients using a targeted next-generation sequencing hotspot panel spanning 65 oncogenes and tumour suppressor genes.

Methods: Pancreatic cancer cells (AsPC1, Capan2) were spiked into 7.5 mL of healthy blood and recovered using a CK+ EpCAM+ CD45- staining panel. Identified cells were then picked using the integrated CytePicker module for mutational analysis using multiplex PCR-based targeted sequencing. 40 pancreatic cancer patients were recruited and blood collected for CTC analysis.

Results: The CTC platform was able to recover 90.9% of spiked-in pancreatic cancer cells. Single cells were picked and confirmed using mutational profiling. CTCs were detected in 77.5% (31/40) pancreatic cancer patients with higher number of CTCs found in patients with more advanced stages. Mutations in KRAS was observed, as expected in pancreatic cancer patients.

Conclusions: CTCs were identified, picked and confirmed using mutational profiling. CTCs were found in pancreatic cancer patients and correlated with clinical stage. Matched patient tissue mutational profiling is currently underway. CTCs presents a potential non-invasive method to identify actionable mutations in cancer patients.

Time-resolved phosphoproteome and proteome analysis reveals kinase signaling on master transcription factors during myogenesis

Di Xiao[1,2], Marissa Caldow[3], Hani Jieun Kim[1,2], Ronnie Blazev[3], Rene Koopman[3], Deborah Manandi[1], Benjamin L.Parker[3], Pengyi Yang[1,2]

[1]Computational Systems Biology Group, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW 2145, Australia
[2]Charles Perkins Centre, School of Mathematics and Statistics, The University of Sydney, Sydney, NSW 2006, Australia
[3]Centre for Muscle Research, Department of Anatomy and Physiology, School of Biomedical Sciences, The University of Melbourne, Melbourne, VIC 3010, Australia

Myogenesis is governed by signaling networks that are tightly regulated in a time-dependent manner. Although different protein kinases have been identified, knowledge of the global signaling networks and their downstream substrates during myogenesis remains incomplete. Here, we map the myogenic differentiation of C2C12 cells using phosphoproteomics and proteomics. From these data, we infer global kinase activity and predict the substrates that are involved in myogenesis. We found that multiple mitogen-activated protein kinases (MAPKs) mark the initial wave of signaling cascades. Further phosphoproteomic and proteomic profiling with MAPK1/3 and MAPK8/9 specific inhibitions unveil their shared and distinctive roles in myogenesis. Lastly, we identified and validated the transcription factor nuclear factor 1 X-type (NFIX) as a novel MAPK1/3 substrate and demonstrated the functional impact of NFIX phosphorylation on myogenesis. Altogether, these data characterize the dynamics, interactions, and downstream control of kinase signaling networks during myogenesis on a global scale.

Multi-omic signatures of immune-checkpoint inhibitor therapy in patients with malignant pleural mesothelioma

Dmitrii, Shek [1,2,3] Scott A Read [1,2,3] Tania Moujaber [3,4] Hema Mahajan [5,6], Won-Hee Yoon [3,4], Joey Lai [2], Brian Gloss [2], Li Ma [2], Hui Emma Zhang [2], Ines Pires da Silva [3,7], Adnan Nagrial [3,4,6], Matteo S Carlino [3,4,6,7], Bo Gao [3,4,6], Golo Ahlenstiel [1,2,3]

[1] *Blacktown Clinical School, Western Sydney University, Sydney, NSW, Australia*
[2] *Westmead Institute for Medical Research, Sydney, NSW, Australia*
[3] *Blacktown Hospital, Sydney, NSW, Australia* [5]
[4] *Westmead Hospital, Sydney, NSW, Australia*
[5] *Institute of Clinical Pathology and Medical Research, Sydney, NSW, Australia*
[6] *Westmead Clinical School, University of Sydney, Sydney, NSW, Australia*
[7] *Melanoma Institute Australia, Sydney, NSW, Australia*

Background. Following the result of Checkmate 743, dual checkpoint inhibition is a standard of care for unresectable malignant pleural mesothelioma (MPM). There is an ongoing need for potential predictive biomarkers for both efficacy and toxicity. To determine how the tumour microenvironment impacts ICI treatment outcomes and irAEs, we have designed a pilot study to determine the role of tumour-infiltrating immune cells on Ipilimumab (IPI) + Nivolumab (NIVO) treatment.

Methods. This pilot trial was conducted as part of a prospective multicentre cohort study (NCT04631731). We performed a VISIUM spatial transcriptomic profiling (10X Genomics) of FFPE tissues. Loupe browser v.6.0.0 and GraphPad v.9 were used for data analysis with ConsensusPath-DB for functional annotation.

Results. We recruited 4 MPM male patients treated with second line IPI (1mg/kg Q6W) + NIVO (3mg/kg Q2W) and median age of 74.5 years. Patients were encoded as A1, B1, C1 and D1. Recent CT scans showed complete response and stable disease in patients A1 and C1 respectively with disease progression in B1 and D1 (both deceased by the time of this report). While being on treatment A1 and D1 developed G3 pneumonitis, C1 was admitted to hospital for G4 hepatitis with B1 had no registered toxicity. Our results established that tumours of non-responders expressed higher expression of inhibitory checkpoint TIM-3 on effector immune cells (NK and T). Moreover, the functional annotation of differentially expressed genes established that complement cascade was upregulated in tumour of responders as compared to non-responders. Regarding signatures of irAEs we determined that B1 had higher number of M2 macrophages and higher expression of HLA genes which could impact its resistance to immune-related toxicity.

Conclusions. Herein we present pilot results of NCT04631731 using cutting-edge spatial analysis in a real-world cohort of patients with cancer. Further validation of established genetic signatures of ICI is currently ongoing.

Ellipse representation for detecting changes in expression and splicing in colorectal cancer

Enikő Regényi [1], Christof Schütte [1,2], Vikram Sunkara [2]

[1] *Department of Mathematics and Computer Science, Freie Universit¨at Berlin, Germany*
[2] *Explainable A.I. For Biology, Zuse Institute Berlin, Germany*

Background: It is generally accepted as fact that the transcriptional landscape of a cell is synonymous with its function. Recent developments in scRNA-seq have made it possible to extract spliced and unspliced transcripts. This high resolution data allows studying the transient states between phenotypes on this landscape. These states highlight driver genes of phenotypic changes which is currently not being exploited due to its complex presentation in the data.

Method: We propose fitting ellipses to the spliced and unspliced counts of individual genes. This uses up to fourth order moments to construct its representation of the data. We then derive two statistics: the overlap between ellipses, which detects change in expression; and the angle between the two major axes, which detects change in splicing. We applied this analysis on a colorectal cancer dataset to analyse the differences between the healthy and the diseased phenotypes.

Results: After filtering, we used 213 genes as input, and found 23 and 19 to be differentially expressed in patients 1 and 2 respectively. We calculated the overlap for all 213 genes, and found that half of the DEGs were within the first quantile of the overlap distribution. We then looked at genes that were not differentially expressed, but had a small overlap, and found that 12 and 10 of those had an angle larger than 10 degrees (which was ~9 fold increase in angle), indicating a significant change in splicing. These include several mitochondrial genes, which are well established to partake in the development of cancer and drug resistance.

Conclusion: Change in phenotype can be induced by change in expression and also by change in splicing. The latter is often neglected, in part due to technical challenges. However, with ellipse representations, detection of changes in splicing in cancer becomes possible.

Identifying universal molecular regulators of 'clonal wave' properties

Esmaeel Azadian [1, 2, 3], Tom Weber [1], Shalin Naik [1, 2, 3]

[1] *Immunology Division, Walter and Eliza Hall Institute, Parkville, VIC, Australia,*
[2] *Department of Medical Biology, University of Melbourne, Parkville, VIC, Australia,*
[3] *Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, VIC, Australia*

During mitosis, a single cell divides into two genetically identical daughter cells which then through successive divisions can create a population of cells termed a "clone". Molecular and functional clonal heterogeneity within a population of seemingly identical cells has been known for decades. The number of cells a clone will produce (clone size) over time (timing), i.e. its 'clonal wave', can vary between the clones of a given cell type. Some clones expand and contract in a short period of time, some remain quiescent for a long time but then expand to become the dominant clone, out-competing others. These distinct clonal wave properties are critical to both normal tissue and cancer development. Importantly, several studies have shown that clonal siblings are highly concordant in their clonal wave behaviour even when placed in separate parallel experiments under similar experimental settings. These observations suggest the existence of intrinsic program(s) in ancestors that dictate similar behaviour amongst clonal siblings i.e., clonal imprinting of clonal wave properties. However, while there have been decades of research regarding the molecular regulators that govern cell division and death, little is known of the mechanisms that guide the timing of such regulators in a clone-intrinsic fashion. Studying clonal wave regulator(s) requires methodologies that provide information at both single cell and clonal levels. Here, I will discuss my experimental plans to address clonal wave properties in several cell types/lines and to assess whether these properties are programmable and controlled by a putatively universal set of regulators. Finding these regulators can help i) answer longstanding and fundamental questions of how clonal waves contribute to the dynamic nature of tissues, ii) inform the underlying genetic/epigenetic programs that dictate which clones become dominant in cancer, iii) identify evolutionarily conserved programs, and v) introduce new targets for medicine (e.g., tissue regeneration, cancer).

Investigating the cellular and AR-activity landscape of treatment-naïve localised prostate cancer using single-cell RNA-seq

Eva Apostolov[1,2], Dan Roden[1,2], Holly Holliday[1,2], Kate Harvey[1], Sunny Wu[1,2], Nenad Bartonicek[1,2], Luciano Martelotto[3,4], Phillip Stricker[1,5], Luke Selth[6], James Kench[1,7], Anthony Joshua[1,5], Lisa Horvath[1,2,8], Alex Swarbrick[1,2]

[1]Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
[2]St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, NSW, Australia
[3]South Australian immunoGENomics Cancer Institute, Adelaide, SA, Australia
[4]The University of Adelaide, Adelaide, SA, Australia
[5]St Vincent's Hospital, Darlinghurst, NSW, Australia
[6]Flinders Health and Medical Research Institute and Flinders Centre for Innovation in Cancer, Flinders University, Bedford Park, SA, Australia
[7]Chris O'Brien Lifehouse, Department of Medical Oncology, Sydney, NSW, Australia
[8]Royal Prince Alfred Hospital, Department of Tissue Pathology and Diagnostic Oncology, Camperdown, NSW, Australia

Prostate cancer (PCa) is a multifocal disease with diverse clinical presentation and outcome. Stratification criteria are heavily reliant on cancer cell morphology, rather than the broader tumour microenvironment (TME). Current prognostic strategies do not incorporate the biology of PCa and cannot accurately predict outcome. Profound cellular and molecular heterogeneity have hindered the discovery of clinically relevant molecular subtypes.

The androgen receptor (AR) is the main disease driver in PCa. Notably, AR is expressed in both the prostatic epithelium and stroma and it exhibits lineage-specific activity (oncogenic and protective, respectively). Most of our knowledge on AR signalling is derived from low resolution/throughput studies that cannot accurately assess the contribution of each lineage, or AR heterogeneity within lineage. We hypothesised that elucidating PCa cellular composition and AR signalling at single-cell resolution will lead to novel insights into PCa biology, clinical behaviour and risk stratification.
We generated a single-cell RNA-seq atlas of 24 treatment-naïve localised PCa, encompassing a broad clinical-risk range. Multi-region analysis of tumour and tumour-adjacent tissue provided a broad overview of the TME, and detailed cellular phenotyping revealed remarkable insights into PCa cellular and molecular heterogeneity, including the detection of a population of fibroblasts unique to tumour-adjacent tissue, and subsets of luminal cells with differential AR activity.

The investigation of the breadth and diversity of AR activity within and across cell types is ongoing, and we have generated complementary multiome (scRNAseq + scATACseq) and spatial transcriptomics data. Our dataset will be used to deconvolute publicly available clinical gene expression datasets to assess associations of cellular heterogeneity with clinical parameters. By adopting an integrated approach to the analysis of PCa in the context of its TME and the involvement of the AR, we hope to raise new hypotheses about PCa biology and gain insight into inherently aggressive PCa phenotypes.

Single-nucleus ATAC-seq elucidates major modules of gene regulation in the development of non-alcoholic fatty liver disease

Fumihiko Takeuchi

National Center for Global Health and Medicine, Tokyo, Japan

Background: Non-alcoholic fatty liver disease (NAFLD) develops from fatty liver to steatohepatitis during which multiple cell types may play different roles. Aiming to understand tissue composition of cell types, their gene expression and global gene regulation in the development of NAFLD, we performed single-nucleus and bulk ATAC-seq on the liver of rats fed with a high-fat diet.

Results: By machine learning, we divided global gene expression into modules, such that transcription factors in a module regulate a set of genes in the same module. Consequently, many of the modules rediscovered known regulatory relationship between the transcription factors and biological processes. For the discovered biological processes, we searched core genes, which were defined as genes central regarding co-expression and protein-protein interaction. A large part of the core genes overlapped with previously implicated NAFLD genes.

Conclusions: Single-nucleus ATAC-seq combined with data-driven statistical analysis help elucidate the global gene regulation in vivo as a combination of modules and discover core genes of the relevant biological processes.

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#fail or #success? Pitfalls and pointers for HTO demultiplexing of scRNA-seq data

George Howitt [1], Jovana Maksimovic [1], Yuzhou Feng [1], Lucas Tobar [1], Steven Stacker [1], Dane Vassiliadis [1], Mark Dawson [1], Sarath Ranganathan [2], Shivanthan Shantikumar [2], Melanie Neeland [2], Alicia Oshlack [1]

[1] Peter MacCallum Cancer Centre, Parkville, Australia,
[2] Murdoch Children's Research Institute, Parkville, VIC, Australia

Hashtag oligos (HTOs) are widely used in single cell sequencing experiments to tag samples prior to pooling, allowing for a multiplexed experimental design that reduces costs and minimises batch effects. HTOs are sequenced along with the RNA and in-silico demultiplexing is required to associate individual cells back to their original samples. Unidentified or misidentified cells can cause issues in downstream analysis.

We investigate the best approaches for performing HTO demultiplexing in a variety of scenarios where we have a range of different tagging qualities. We begin by describing some basic quality control analysis that can be used to assess the quality of HTO counts data. We then perform a comparative analysis of five hashtag demultiplexing tools: hashedDrops, HTODemux, GMM-Demux, deMULTIplex and BFF. We analyse two data sets, one consisting of 24 bronchoalveolar lavage samples taken from paediatric patients which uses Totalseq-A antibody-derived HTOs, and a second consisting of three genetically distinct human lung cancer cell lines tagged with MULTI-seq lipid-modified oligos. With both data sets, we perform genetic demultiplexing on the RNA counts using vireo, which provides a ground truth for sample identification to assess the performance of the HTO demultiplexing tools. We find that all tools perform well on high quality data, but the performance of some methods deteriorates when the tagging is of lower quality. Specifically, methods that make assumptions about the counts distributions perform poorly on lower quality data where these assumptions don't hold.

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High-plex spatial proteomic profiling of immunotherapy response groups in head and neck cancer identifies tissue signatures associated with therapy response

Habib Sadeghirad [1], Ning Liu [2], James Monkman [1], Chin Wee Tan [2], Caroline Cooper [3], Ken O'Byrne [3], Melissa Davis [2], Brett Hughes [4], Arutha Kulasinghe [1]

[1] *University of Queensland Diamantina Institute, The University of Queensland, Brisbane, QLD, Australia.*
[2] *The Walter and Eliza Hall Institute*
[3] *The Princess Alexandra Hospital, Brisbane, QLD, Australia*
[4] *The Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia*

Background: Head and neck squamous cell carcinoma (HNSCC) is a type of cancer that originates in various anatomical regions of the head and neck region and is typically diagnosed at an advanced stage of disease. Immunotherapy has shown promising results for patients with recurrent or metastatic (R/M) disease, but only a small percentage of patients benefit from the treatment. Therefore, novel predictive biomarkers informative of therapy response are required.

Methods: In this retrospective study, 21 formalin-fixed paraffin-embedded (FFPE) tissue samples from immunotherapy-treated patients were collected at the Royal Brisbane and Women's Hospital (RBWH). Spatially resolved tissue compartment-specific analyses of protein biomarkers were done by a 78-plex immune-oncology panel, including cell death, immune activation status, immune cell typing, immune cell profiling, PI3K/AKT signalling, Pan-tumour, and IO drug target panels, using the Nanostring Technologies Digital Spatial Profiler (DSP). Survival analyses based on Response Evaluation Criteria in Solid tumours (RECIST) were performed against tissue compartment specific protein expression.

Results: Our data unravelled significant differentially expressed proteins in tumour and stromal regions in patients with partial response (PR) versus those with progressive disease (PD). In the tumour compartment, patients with PR had a higher expression of protein biomarkers, such as PD-1, PD-L1, CD68, ER-alpha, HLA-DR, IDO-1, and NY-ESO-1, while patients with PD showed higher expression levels of VISTA, CD66b, CD44, CD127, and CD45RO. In the stromal compartment, patients with PR had a higher expression of PD-1, PD-L1, ER-alpha, HLA-DR, and CD68, while those with PD had an increased expression of VISTA, BIM, BAD, and CD44. **Conclusion**: There is an increasing need to comprehensively profile tumour tissues from HNSCC using high-plex automated imaging and tissue profiling methodologies. Here, we demonstrate a whole-slide, multi-region study to demarcate tissue signatures associated with therapy response.

Comprehensive Characterisation of Fetal and Mature Retinal Cell Identity to Assess the Fidelity of Retinal Organoids

Hani Jieun Kim[1-3], Michelle O'Hara-Wright[3,4], Daniel Kim[1,3], To Ha Loi[3,6], Benjamin Y. Lim[4], Robyn V. Jamieson[5,6], Anai Gonzalez-Cordero[3,4], Pengyi Yang[1-3]

[1]Computational Systems Biology Group, Children's Medical Research Institute, The University of Sydney, Westmead, 2145, NSW, Australia
[2]School of Mathematics and Statistics, The University of Sydney, Sydney, NSW 2006, Australia
[3]School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, NSW 2006
[4]Stem Cell Medicine Group, Children's Medical Research Institute, The University of Sydney, Westmead, 2145, NSW, Australia
[5]Specialty of Genomic Medicine, Faculty of Medicine and Health, University of Sydney, Westmead, 2145, NSW, Australia
[6]Eye Genetics Research Unit, Children's Medical Research Institute, Sydney Children's Hospitals Network, Save Sight Institute, The University of Sydney, Westmead, 2145, NSW, Australia

Characterizing cell identity in complex tissues such as the human retina is essential for studying its development and disease. While retinal organoids derived from pluripotent stem cells have been widely used to model development and disease of the human retina, there is a lack of studies that have systematically evaluated molecular and cellular fidelity of the organoids derived from various culture protocols in recapitulating their in vivo counterpart. To this end, we performed an extensive meta-atlas characterisation of cellular identities of the human eye, covering a wide range of developmental stages. The resulting map uncovered previously unknown biomarkers of major retinal cell types and those associated with cell-type specific maturation. Using our retinal cell identity map from the fetal and adult tissues, we systematically assessed the fidelity of the retinal organoids to mimic the human eye, enabling us to comprehensively benchmark the current protocols for retinal organoid generation.

UTS Single Cell Technology Facility: Providing personalised solutions for single cell multiomics

Holly C.L Pearson[1], David Gallego Ortega[1]

[1]*School of Biomedical Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia*

Since the introduction of the first single cell RNA sequencing (scRNA-seq) technique in 2009, single cell technologies have enabled researchers to catalogue the cell composition of a rapidly increasing number of tissues, characterising rare and heterogeneous cell populations with unprecedented detail. This has revolutionised biological analyses, transforming studies in many fields of biology and medicine, including cancer, neuroscience, immunology and development. The exciting potential of single cell technology led to the rapid expansion of this field over the last decade, making it possible to not only interrogate the transcriptome of single cells, but the genome, epigenome, and proteome. Now, there is also an increasing demand to combine these methods for single cell multiomic approaches. While single cell technology has generated a lot of excitement over the years, its rapid uptake within Australia has been limited by not only high costs but also the need for specialised facilities and equipment, in-depth molecular knowledge, and time-consuming optimisation. Knowing this, and with the aim to increase the accessibility of single cell technology to researchers and industry partners across Australia, the Single Cell Technology Facility at the University of Technology Sydney (UTS) was created. Our facility brings together leading single cell platforms and the latest technology in a laboratory dedicated for single cell workflows. We offer a range of expert services for researchers and industry partners, including experimental design and end-to-end workflows for single cell analyses from cell sorting, capture, and barcoding to library preparation. Here, we highlight some of our recent work to showcase the potential of our facility within the broader single cell community.

Defining the immune milieu of pancreatic adenocarcinoma using single-nuclei RNA sequencing

Hue M. La [1], Fernando J. Rossello [1], Luciano G. Martelotto [2], Jacek Marzec [1], Amber Jones [1], Anna Leichter [1], Sean M. Grimmond [1]

[1] University of Melbourne Centre for Cancer Research, Victorian Comprehensive Cancer Centre, Melbourne, 3000, VIC, AUSTRALIA.
[2] Adelaide Centre of Epigenetics, South Australian immunoGENomics Cancer Institute

Pancreatic Adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers in Western societies with a 5-year survival rate of 7%. Current treatments are limited and ineffective, partly due to complex cellular compositions and interactions between tumours and its microenvironment that are largely undefined. Therefore, there is an urgent need to better understand the molecular pathology and oncogenesis of PDAC to improve treatment options for cancer patients and to develop novel therapeutic strategies.

In a multi-national effort, our group have previously performed a large scale genomic/transcriptomic/epigenomic analyses of >1000 pancreatic adenocarcinomas, expanding the repertoire of driver mutations involved in this disease to more than 60. However, standard bulk sequencing approach does not resolve intra-tumour heterogeneity, cancer cell states, cellular composition and cell-specific expression signature.

In this current work, we performed single nucleus RNA and ATAC high throughput sequencing, from the same sample preparation, of 30 extensively characterised PDAC - part of Australia ICGC (International Cancer Genome Consortium) program - using the 10X Genomics platform. Using a multimodal integrative approach, we characterised the transcriptome and epigenome of each tumour sample within the context of the entire cohort. This study will address the limitations from previous bulk sequencing analyses to better define tumour classification and composition, immune milieu, stromal composition plus cell-specific activation states during PDAC progression. In this instance, we characterise the lymphoid and myeloid component of the PDAC, including differences between malignant cell subtypes and histopathological settings. In depth analyses of immune subtypes in different cancer cell states will improve classification of tumour composition, which is key to therapeutic strategies that target each cancer subtype/state effectively.

Using Single-cell Gene Expression to Develop Treatment Response Predictions in Multiple Myeloma

HuiQi Hong [1], Jonathan Scolnick [1], Stacy Xu [1], Grant Roy [1], Sanjay De Mel [2], Cinnie Yentia Soekojo [2], Wee Joo Chng [2,3]

[1] Singleron Biotechnologies Pte Ltd, Singapore
[2] Department of Haematology Oncology, National University Cancer Institute, Singapore;
[3] Department of Medicine, National University of Singapore,

The promise of precision medicine in oncology is to identify the ideal treatment for each patient, eliminating failed treatment cycles and reducing treatment burdens on patients and payors. While gene sequencing can predict effective treatment options in some cases, studies have estimated that only 10-15% of cancer patients are treated with genotype-matched drugs. Gene expression is an attractive alternative to predicting therapeutic response because it provides a direct readout of dysregulated cellular activities. However, the use of bulk gene expression to predict therapeutic response is hampered by tumor heterogeneity as a biopsied tumor contains multiple cell types and different tumor clones. Bulk analysis only generates an averaged gene expression signal across all of the cells, leading to a decreased sensitivity for identifying biomarkers found only in specific cell subgroups. Recently, single-cell RNA expression analysis technologies have grown in popularity. These technologies enable finer characterization of tumors by making gene expression measurements from each discrete cell within the sample and, therefore, hold the promise of increasing the power of gene expression measurements for developing therapy response prediction tools.

Here we combine single-cell gene expression analysis with Singleron's MapResponse™ machine-learning algorithms to develop a predictive classifier for patient response to Daratumumab treatment in multiple myeloma (MM). Starting with publicly available data (Cohen et al., Nat. Medicine, 2021), we developed an improved patient response classifier that accurately predicted the response of 94% of subjects in the published study. In addition, the classifier accurately predicted response in an independent cohort of Daratumumab-treated patients. In contrast, the classifier performed poorly in a cohort of patients who did not receive Daratumumab. These data suggest the classifier specifically predicts Daratumumab response and is not a general prognostic signature. These early findings highlight the potential value of combining single-cell RNA sequencing with machine-learning methods such as MapResponse™ to bring precision medicine to more patients.

Mapping Rare Inflammatory Bowel Disease Pathologies at Single Cell Resolution

Jacqueline L. E. Tearle[1,2], Georgina Hold[3], Paris Tavakoli[3,4], Simon Ghaly[4], Kylie R. James[1,2]

[1]*Garvan Institute of Medical Research, Darlinghurst, Australia*
[2]*School of Medical Sciences, UNSW Sydney, Australia*
[3]*St George and Sutherland Clinical School, Faculty of Medicine and Health, UNSW Sydney, Australia*
[4]*St Vincent's Clinical School, UNSW Sydney, Australia*

Colorectal cancer (CRC) is the second leading cause of death due to cancer in Australia. For patients with inflammatory bowel disease (IBD) linked to primary sclerosing cholangitis (PSC; a rare progressive liver disease), the risk of developing CRC is 6 times higher than that of the general public, and 4 times higher than that of patients presenting with IBD alone. It is now accepted that CRC development is underpinned by dysregulation of the immune-microbiota axis, as well as genetic predisposition and environmental factors. However, the immunological interactions driving increased and differential CRC risk in IBD and PSC-IBD patients are incompletely understood and could offer new insights into CRC risk stratification and earlier diagnosis. To address this, here we compare the intestinal immune cell and microbial landscapes in PSC-IBD and ulcerative colitis. We perform parallel single cell RNA, single cell immune receptor and bacterial metagenomic sequencing on mucosal pinch biopsies from these patient cohorts. By applying cutting-edge computational analyses, we will define at high resolution the regional and disease-specific immune, epithelial, and microbial landscapes. Early and emerging insights from this multi-omic study show distinct cell signatures between patient cohorts with potential relevance in elucidating the differential CRC risk and pathology of this rare IBD subtype.

No time to retire: bulk buying in the age of single cell

Jarny Choi [1], Suzanne Butcher [1], Yidi Deng [1], Christine Wells [1]

[1] *University of Melbourne, Australia*

Single cell gene expression datasets are being produced at an unprecedented rate. Human Cell Atlas Data Portal hosts 260+ projects, for example, spanning more than 27 million cells from various human tissues. Many of these datasets can act as references for query data, and even fast automated cell type classifications are possible by uploading query data and clicking a few buttons online (celltypist.org). Can bulk data still act as useful references in this age of single cell atlases? We compared the results of querying several single cell datasets for cell type classification, using either single cell or bulk atlases as references. We show that bulk atlases shine when exploring more nuanced biology, such as benchmarking in vitro systems where various experimental factors are important. We have built a prototype online tool for querying single cell data against bulk integrated atlases at Stemformatics.org.

A comparison of marker gene selection methods for single-cell RNA sequencing data

Jeffrey Pullin[1,2,3], Davis McCarthy[1,2,3]

[1]*Bioiformatics and Cellular Genomics, St Vicent's Institute of Medical Research, Fitzroy, Australia*
[2]*School of Mathematics and Statistics, Faculty of Science, University of Melbourne, Parkville, Australia*
[3]*Melbourne Integrative Genomics, Faculty of Science, University of Melbourne, Parkville, Australia*

The development of single-cell RNA sequencing (scRNA-seq) has enabled scientists to catalogue and probe the transcriptional heterogeneity of individual cells in unprecedented detail. A common step in the analysis of scRNA-seq data is the selection of so-called marker genes, most commonly to enable annotation of the biological cell types present in the sample. In this paper we benchmarked 56 computational methods for selecting marker genes in scRNA-seq data. The performance of the methods was compared using 10 real scRNA-seq datasets and over 170 additional simulated datasets. Methods were compared on their ability to recover simulated and expert-annotated marker genes, the predictive performance and characteristics of the gene sets they select, their memory usage and speed and their implementation quality. In addition, various case studies were used to scrutinise the most commonly used methods, highlighting issues and inconsistencies. Overall, we present a comprehensive evaluation of methods for selecting marker genes in scRNA-seq data. Our results highlight the efficacy of simple methods, especially the Wilcoxon rank-sum test, Student's t-test, and logistic regression.

Dual single-cell RNA sequencing to characterize molecular mechanisms of the macrophage zinc toxicity antimicrobial response against intracellular *Escherichia coli*

Jessica B. von Pein[1,2], Stacey B. Andersen[3,4], Jun Xu[3], Nicholas D. Condon[1], Nathan Palpant[1], Minh-Duy Phan[5,6], Christian Nefzger[1], Claudia J. Stocks[1,2,5], Ronan Kapetanovic[1,2,5], Mark A. Schembri[5,6] and Matthew J. Sweet[1,2,5]

[1]*Institute for Molecular Bioscience (IMB), The University of Queensland*
[2]*IMB Centre for Inflammation and Disease Research, The University of Queensland*
[3]*Genome Innovation Hub, The University of Queensland*
[4]*UQ Sequencing Facility, The University of Queensland*
[5]*Australian Infectious Diseases Research Centre, The University of Queensland*
[6]*School of Chemistry and Molecular Biosciences, The University of Queensland*

Urinary tract infections are one of the most common bacterial infections that impact humans, often caused by Uropathogenic E. coli (UPEC). During infection, innate immune cells can manipulate trace element availability to either starve or toxify microorganisms as antimicrobial strategies. Macrophages can deploy zinc toxicity against intracellular bacteria during infection, but molecular mechanisms that facilitate this pathway are poorly understood. Here, we investigate the mechanisms involved in the zinc toxicity response in human macrophages using fluorescent reporter E. coli that constitutively expresses GFP and expresses mCherry under zinc stress. The zinc toxicity response in human monocyte-derived macrophages (HMDM) was variable between monocyte donors, and between individual macrophages across whole populations. This heterogeneity was exploited for gene discovery, through the development of a 10X Genomics single-cell RNA sequencing platform to assess both host and bacterial transcriptomes associated with the HMDM zinc toxicity response. We utilised feature barcode enrichment to detect GFP and mCherry transcripts from reporter E. coli within single macrophages. This allowed us to assess HMDM transcriptomes and genes associated with the zinc toxicity response. These methods confirmed a small population of HMDM harboured intracellular zinc-stressed E. coli, thereby validating the heterogeneity of the zinc toxicity response across whole HMDM populations. Clustering and differential gene expression analyses confirmed that a macrophage sub-population enriched for zinc-stressed E. coli engage in zinc ion homeostasis genetic pathways and identified potential regulators of the zinc toxicity response. These include specific metal-binding metallothioneins (MT1H, MT1G), a zinc transporter (SLC30A4), and novel candidates with unknown roles in zinc biology (S100A10 and TPT1). Ongoing studies utilising an inducible overexpression system in macrophage-like THP-1 cells seek to characterise the role(s) of these candidate genes in the macrophage zinc toxicity response. Ultimately, this knowledge may facilitate the development of host-directed therapies for the treatment of antibiotic-resistant bacterial infections.

Poster Session II

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decode science

Solving the three of transcriptional events and decision-making for neural crest lineage

Igor Adameyko[1], Maria Eleni Kastriti [1], Louis Faure [1], Dorothea Vin Ahsen [1]

[1] Center for Brain Research, Medical University of Vienna, Vienna, Austria

Neural crest cells (NCCs) represent a 4th germ layer in an embryo. Being born at the dorsal neural tube, these neuroepithelial cells delaminate and migrate towards multiple destinations, where they give rise to dozens of cell types. A portion of the NCCs settles on the embryonic nerves to ride them and reach distant destinations, where nerve-associated now Schwann cell precursors (SCPs) give rise to late neural crest-derived cell types. The true identity and multipotency of SCPs always stayed puzzling.

Here we focused on questioning if nerve-associated SCPs are actually neural crest cells living in the embryonic nerves. The answer is not yes or no. Using Smart-seq2, we created the deepest atlas (7000 genes/cell) of the neural crest and SCP lineage till postnatal stages. It turned out that SCPs and neural crest cells (NCCs) are highly similar, but not the same, and both converge into a common transcriptional “hub” state characterized by multiple biases towards downstream fates. Overall, we realized that nerve associated cells represent a special state of NCCs, or NCC-like cells. Overall, SCPs turned out to be similar to the crest because they express classical NCC gene regulatory network (Sox10, FoxD3, Tfap2a/b, Ets1 etc.). However, they have something in addition to it: high levels of Sox8, Itga4 and some other genes. SCPs and “hub” cell in general are highly heterogeneous and show the presence of different competing programs driving them into a spectrum of fates. Late NCCs blend into a “hub” population, which is mainly SCP-based. Note that the tree of transcriptional events might differ from clonal trees, also in our case and mainly because many NCCs can “tunnel” or bypass the “hub” state when they differentiate into terminal fates. Still, SCPs occupy the intercalating “hub” position, which means that SCPs evolved as long-living crest in the nerves.

Multi-omic and spatial dissection of immunotherapy response groups in non-small cell lung cancer (NSCLC)

James Monkman [1], Ahmed Mehdi [2], Nicholas Matigian [2], Marie Cumberbatch [3], Milan Bhagat [3], Rahul Ladwa [4], Scott N Mueller [5], Mark N Adams [6], Ken O'Byrne [4], Arutha Kulasinghe [1]

[1] *The University of Queensland Diamantina Institute, UQ, Brisbane, QLD, Australia*
[2] *QFAB Bioinformatics, UQ, Brisbane, QLD, Australia*
[3] *Tristar Technologies Group, USA*
[4] *The Princess Alexandra Hospital, Brisbane, QLD, Australia*
[5] *The University of Melbourne, Vic, Australia*
[6] *Queensland University of Technology, Brisbane, QLD, Australia.*

Introduction: Lung cancer is the leading cause of cancer-related mortality globally with a 5-year survival ~20%. Whilst immunotherapies have shown durable benefit, it appears that these are only effective in a subset of non-small cell lung cancer (NSCLC) patients. The composition and activation status of the cellular milieu contained within the tumour microenvironment(TME) is becomingly increasingly recognised as a driving factor dictating response to immunotherapies.

Methods: Here, we employed multiplex IHC(mIHC), and digital spatial profiling(DSP) to capture the targeted immune proteome and transcriptome of tumour and TME compartments from ICI-treated(n=41) and standard of care(n=47) NSCLC patient cohorts.

Results: We demonstrate by mIHC that the interaction of CD68+ macrophages with PD1+, FoxP3+ cells is enriched in ICI refractory tumours (p=0.012). Patients sensitive to ICI therapy expressed higher levels of IL2 receptor alpha (CD25, p=0.028) within the tumour compartments, which corresponded with increased IL2 mRNA (p=0.001) within their stroma. IL2 mRNA levels within the stroma positively correlated with the expression of pro-apoptotic markers cleaved caspase 9 (p=2e-5) and BAD (p=5.5e-4) and negatively with levels of memory T cells (CD45RO) (p=7e-4). Immuno-inhibitory markers CTLA-4 (p=0.021) and IDO-1 (p=0.023) were supressed in ICI-responsive patients. Tumour CD44 (p=0.02) was depleted in the response group and corresponded inversely with higher stromal expression of one of its ligands, SPP1 (osteopontin,p=0.008). Cox survival analysis indicated tumour CD44 expression was associated with poorer prognosis (HR=1.61,p=0.01), consistent with its depletion in ICI sensitive patients. The SOC cohort paralleled similar roles for immune checkpoints and pro-apoptotic markers, with LAG3 (HR=3.81,p=0.04) indicating poorer outcome, and BIM (HR=0.16,p=0.014) with improved outcome

Conclusions: Through multi-modal approaches, we have dissected the characteristics of NSCLC treatment groups and provide evidence for the role of several markers including IL2, CD25, CD44 and SPP1 in the efficacy of current generations of ICI therapy.

STOmics identifies response associated cellular neighbourhoods in Hepatocellular Carcinoma

Jennifer Currenti[1,2], Rhea Pai[1,2], Saurabh Gupta[1,2], Ziyi Li[3], Ashley Soet[1,4], Liang Qiao[5], Bicheng Yang[6], Louise Winteringham[1], Shirely Go[4], Florent Ginhoux[3,8,9], Peter Leedman[1], Michael Wallace[4,7], Luciano G. Martelotto[10], Jacob George[5], Ankur Sharma[1,2,11,12*]

[1]Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands, Australia
[2]Curtin Medical School, Curtin Health Innovation Research Institute, Curtin University, Bentley, Australia
[3]Shanghai Institute of Immunology, Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[4]Medical School, University of Western Australia, Crawley, Australia
[5]Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, Sydney, NSW, Australia
[6]BGI-Australia, 300 Herston Rd, Herston, Queensland, 4006, Australia
[7]WA Liver Transplant Service, Sir Charles Gairdner Hospital, Perth, Australia
[8]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A∗STAR), 138648 Singapore
[9]Gustave Roussy Cancer Campus, Villejuif 94800, France
[10]South Australian Immunogenomics Cancer Institute, The University of Adelaide
[11]Institute of Molecular and Cellular Biology (IMCB), Agency for Science, Technology and Research (A∗STAR), Singapore
[12]Lead contact [*] Correspondence: ankur.sharma@perkins.org.au (A.S.)

Hepatocellular carcinoma (HCC) is the fastest growing cause of cancer-associated mortality in Australia. Despite curative surgery and ablation, ~50% of patients will relapse within five years with immunotherapy for advanced HCC only having a response rate of ~30%. Consequently, there is a dire need to identify predictive biomarkers and new therapies to improve outcomes. Our laboratory recently discovered the presence of fetal-like cells in the HCC tumor microenvironment and termed this phenomenon as an oncofetal ecosystem. More importantly, these oncofetal cells orchestrate an immunosuppressive microenvironment in HCC. Therefore, we hypothesized that tumors with high proportions of oncofetal cells may respond better to immunotherapy, providing a biomarker for therapeutic stratification. To investigate this, we employed the state-of-the-art spatial transcriptomics method STOmics, which provides 'single cell resolution' with a spot size of 200nm. We optimized the STOmics wet-lab workflow to 'enrich' for biologically relevant genes and thereby, improved cellular annotations and allow for the discovery of new biology. Use of this novel high-resolution technology allowed us to identify niches within the tumor microenvironment of HCC that were enriched for fetal-like cells (oncofetal niches). Crucially, we observed significant co-localization between an oncofetal microenvironment and immunotherapy response associated cells in HCC patients. Taken together, we were able to identify and quantify oncofetal biomarkers within HCCs that predict therapy response. This work provides new insights into the potential implications of oncofetal cells as a biomarker of therapy response in HCC.

Single-cell RNAseq of amniotic fluid: a sneak peak into fetal development and disease

Jovana Maksimovic [1,5,6], Calandra Grima [1], Andreas Halman[1], Shalini Guleria [3], Sally Beard [2,8], Natalie Hannan [2,8], Bhupinder Pal [3], Lisa Hui [2,4,8], Alicia Oshlack [1,6,7]

[1] Peter MacCallum Cancer Centre, Computational Biology Program, Parkville, Australia
[2]Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Australia
[3]Oliva Newton-John Cancer Research Institute, Single Cell Cancer Genomics Laboratory, Heidelberg, Australia
[4]Murdoch Children's Research Institute, Reproductive Epidemiology Group, Parkville, Australia
[5]Murdoch Children's Research Institute, Respiratory Group, Parkville, Australia
[6]The Sir Peter Maccallum Department of Oncology, University of Melbourne, Parkville, Australia
[7]School of Bioscience, University of Melbourne, Parkville, Australia
[8]Department of Perinatal Medicine, Mercy Hospital for Women, Heidelberg, Australia

The study of live, human fetal cells is challenging due to both practical and ethical constraints. Single-cell RNAseq analysis of amniotic fluid samples may provide a unique insight into fetal development and disease by profiling the gene expression of live cells present in the fluid. As a proof of concept, scRNAseq was performed on live cells purified from amniotic fluid collected via amniocentesis from two second trimester pregnancies at high-risk of cytomegalovirus (CMV) infection. Using PCR, one sample was confirmed as CMV positive and the other CMV negative. CMV sequences were only detected in the scRNAseq data of the CMV positive sample. The scRNAseq data also reflected the sex of each foetus based XIST and SRY expression.
After quality control, 1882 intact cells were recovered across the two samples. The cells were annotated using a combination of two different human fetal development references in conjunction with three different tools: Azimuth, SingleR and ProjecTILs. Lung-derived squamous epithelial cells were the most abundant cell type, followed by myeloid and lymphoid cells. Overall, 1245 cells (66%) were consistently annotated as either epithelial cells, immune and blood cells or secretory cells; the consistency of the labels was related to the quality of the cell. Unsupervised clustering of the 1245 consistently labelled cells, revealed 9 sub-clusters within the broad, reference-assigned labels. The marker genes and GO categories for each cluster were also indicative of epithelial, myeloid, lymphoid or secretory lineages. Intriguingly, the CMV positive sample contained a lower proportion of megakaryocytes (MK) than the negative sample, which may be related to its documented inhibition of MK proliferation, differentiation, maturation, and its stimulation of MK apoptosis.

Although laboratory protocols require further optimisation, this unique study demonstrates the feasibility of examining live fetal cells from amniotic fluid using scRNAseq and its potential for clinical and research applications.

Spatial omics reveals differential fibrotic responses to spinal cord injury that are lesion-level-dependent

Laura F. Grice[1,2], Ellen R. Gillespie[1], Duy Pham[2], Quan Nguyen[2], Marc J. Ruitenberg[1]

[1]School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, Brisbane, Australia
[2]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia

BACKGROUND
56% of Australian spinal cord injuries (SCIs) are high-level lesions at or above thoracic level T2, meaning that at least half of all patients are affected by the most severe SCI symptoms. Immune impairment is one pathological feature of high-level SCI, but little is known about how this impacts early inflammation and scarring within the injured site itself. Here we used single-cell and spatial omics to uncover the interplay between SCI lesion level, inflammation, and scar formation.

METHODS AND RESULTS
We have built an immune atlas of >35,000 cells, collected from the spinal cord lesion site during the first week of injury, through single-cell RNASeq. The immune infiltrate was highly heterogenous and dynamic, with 39 different immune cell subtypes and/or transcriptional states identified over this time period. We then mapped the location of cell (sub)types across time, space, and lesion level (i.e. high-level T2 and lower-level T9 injuries) using Visium spatial transcriptomics, finding that the different cell subtypes localised to particular anatomical microdomains within the lesion site. Comparison across injury levels revealed a strong fibrotic signature in high-level T2 injuries, accompanied by a shift in the cellular microenvironment and changes in cell crosstalk, as revealed by spatially-guided cell-cell interaction analysis.

CONCLUSION
By integrating data from independent yet complementary spatial and single-cell technologies, we were able to build a comprehensive spatial atlas of inflammation and fibrosis in acute SCI. We reveal unprecedented diversity of infiltrating immune cells, have mapped their activity to anatomical context, and profiled how these cells contribute to a differential fibrotic signature between lesion levels. This information is valuable for follow-up studies to dissect the role of inflammation in local spinal cord wound healing responses.

Spatially-resolved cell-cell interaction analysis of cancer tissue morphology

Minh Tran[1], Andrew Xu[1], Quan Nguyen[1]

[1]*Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia*

Since crosstalk between cells has direct biological links to tissue homeostasis and disease, spatial analysis of cell-cell interaction (CCI) within the tissue microenvironment is crucial for developing therapeutics. The recent development of highly multiplexed fluorescence imaging, which simultaneously captures many transcripts and/or proteins from a single tissue section, has already provided a wealth of information on the molecular activity of cells within a tissue. We can use this data to explore enrichment of cell type colocalization within tissue microenvironments and understand interactions between cell types via ligand-receptor signalling. Here we present a quantitative Python-based analysis pipeline for studying CCI, using the information obtained by multiplexed imaging. The pipeline enables an entire workflow of analyses, from uncovering spatial CCI via ligand-receptor pairs, through to correlating spatial distributions of cell communities with clinical prognosis. We demonstrate the application of this method for the analysis of cancer tissues across multiple patients. Our pipeline shows high versatility when applied to different spatial transcriptomic (i.e., RNA In situ Hybridisation and CosMx) and proteomic (Imaging Mass Cytometry and Opal Polaris) data modalities. Comparison of the results from our analysis pipeline with pathological annotations revealed high consistency between predictions and annotated histopathological features, and demonstrated high correlation with clinical information. Through the spatial mapping of cell types and interactions across cancer and immune cell populations, we found that crosstalk between cancer cells and CD4 T cells is highly correlated with the microsatellite instability (MSI) status in colorectal cancer. As more powerful multiplexed imaging platforms are emerging, we expect to see the growing needs for our analysis pipeline to unravel spatial CCI.

Defining the fetal gene program at single cell resolution in pediatric dilated cardiomyopathy.

Neda R. Mehdiabadi [1,2,3], Choon Boon Sim [1,2], Belinda Phipson [1,4,5,6], Ravi K. R. Kalathur [1,2], Yuliangzi Sun[7], Celine J. Vivien [1,2], Menno ter Huurne [1,2], Adam T. Piers [1,2], James E. Hudson [8], Alicia Oshlack [1,4,5,9], Robert G. Weintraub [2,6,10], Igor E. Konstantinov [2,6,11], Nathan J. Palpant [7], David A. Elliott [1,2,3,6,12], Enzo R. Porrello [1,2,12,13]

[1] Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne 3052, VIC, Australia.
[2] Melbourne Centre for Cardiovascular Genomics and Regenerative Medicine, The Royal Children's Hospital, Melbourne 3052, VIC, Australia.
[3] Australian Regenerative Medicine Institute, Melbourne 3800, Monash University, VIC, Australia.
[4] Peter MacCallum Cancer Centre, Melbourne 3000, VIC, Australia.
[5] Sir Peter MacCallum Department of Oncology, The University of Melbourne, Victoria 3010, Australia
[6] Department of Paediatrics, The University of Melbourne, Melbourne 3052, Victoria, Australia.
[7] Institute for Molecular Bioscience, The University of Queensland, Brisbane 4072, QLD, Australia.
[8] QIMR Berghofer Medical Research Institute, Brisbane 4006, QLD, Australia.
[9] School of Biosciences, The University of Melbourne, Melbourne, Victoria, 3010, Australia.
[10] Department of Cardiology, The Royal Children's Hospital, Melbourne 3052, VIC, Australia.
[11] Department of Cardiac Surgery, The Royal Children's Hospital, Melbourne 3052, VIC, Australia.
[12] Novo Nordisk Foundation Center for Stem Cell Medicine, Murdoch Children's Research Institute, Melbourne 3052, VIC, Australia.
[13] Department of Anatomy and Physiology, School of Biomedical Sciences, The University of Melbourne, Melbourne 3010, VIC, Australia.

Dilated cardiomyopathy (DCM) is a disease of the heart muscle characterized by enlargement of the heart's chambers, ventricular wall thinning and impaired contractility. Heart transplantation is the only therapeutic option currently available for end-stage DCM. However, it has been suggested that the postnatal failing heart reverts to foetal gene expression pattern as an adaptive process to protect the stressed heart during cardiac remodelling. We assessed this hypothesis by performing single nuclei RNA sequencing to examine gene expression profiles in foetal, paediatric non-diseased and paediatric patients with dilated cardiomyopathy. This comparative study demonstrated that the primary transcriptional responses to DCM are observed in cardiomyocytes and fibroblasts. Furthermore, analysis of cardiomyocyte gene expression profiles suggested that while a fully foetal-like gene expression profile is not observed in DCM patients, a specific sub-set of foetal genes are re-activated in the diseased heart. This work lays the foundation to define the transcriptional networks perturbed in DCM and may serve as a platform to develop targeted therapeutics to improve patient outcome in DCM.

A single cell and spatial atlas of Hepatocellular Carcinoma across aetiologies

Rhea Pai[1,2,#], Saurabh Gupta[1,2,#], Jennifer Currenti[1,2], Ashley Soet[1,3], Ziyi Li[4], Liang Qiao[5], Shirley Go[6], Jordan Crane[1], Louise Winteringham[1], Peter Leedman[1], Florent Ginhoux[4,7,8], Michael Wallace[6], Jacob George[5], Ankur Sharma[1,2,9,10,*]

[1]Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, 6 Verdun Street, Nedlands, Perth, Western Australia 6009, Australia
[2]Curtin Medical School, Curtin Health Innovation Research Institute (CHIRI), Curtin University, Perth, Western Australia 6102, Australia
[3]The University of Western Australia, Perth, Western Australia 6009, Australia
[4]Shanghai Institute of Immunology, Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[5]Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, Sydney, NSW, Australia
[6]WA Liver Transplant Service, Sir Charles Gairdner Hospital, Perth, Australia
*[7]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), 138648 Singapore*
[8]Gustave Roussy Cancer Campus, Villejuif 94800, France
*[9]Institute of Molecular and Cellular Biology (IMCB), Agency for Science, Technology and Research (A*STAR), 138673 Singapore.*
[10]Lead Contact
[#]These authors contributed equally
**Correspondence: ankur.sharma@perkins.org.au*

Hepatocellular carcinoma (HCC) is the leading cause of liver cancer (~90% of total cases) and is triggered by distinct aetiologies. These distinct aetiologies include viral infections (Hepatitis B and Hepatitis C), metabolic factors (Metabolic dysfunction Associated Fatty Liver Disease), alcohol consumption (Alcoholic liver disease) and cirrhosis (Non-alcoholic steatohepatitis). Our laboratory previously identified fetal liver-like transcriptomic signatures in the HCC tumour microenvironment termed as “oncofetal” ecosystem. But it is still unknown how viral or metabolic aetiologies impacts the presence of oncofetal cells, their spatial organisations and immunosuppressive properties of tumour microenvironment. To address this question, we employed 5’ scRNA-seq with TCR and BCR profiling from 23 tumor sections collected from 11 HCC patients. In total, we sequenced ~63,000 cells and identified 31 major clusters in the HCC atlas. Further, we performed spatial transcriptomics by employing Visium technologies to determine the spatial localisation of these cells. Finally, we validated these observations by performing state-of-the-art high-plex in situ analysis by employing CosMx technology. Our data reveals the distinct oncofetal neighbourhoods across HCC aetiologies and disease stages. This work unravels the spatial heterogeneity of HCC in a clinical context, thereby paving the way for precision medicine to guide clinical decisions.

An experimental perspective of the Digital Spatial Profiling (DSP) and Visium technologies

Taopeng Wang[1,2], Kate Harvey[1], John Reeves[1], Daniel Roden[1], Nenad Bartonicek[1], Jessica Yang[1], Ghamdan Al-Eryani[1], Chia-ling Chan[3], Dominik Kaczorowski[3], Joseph Powell[3], Elgene Lim[1,2], Alexander Swarbrick[1,2]

[1]Cancer ecosystems program, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia
[2]School of Clinical Medicine, Faculty of Medicine, UNSW Sydney, NSW 2052, Australia
[3]Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia

Tumours are cellular ecosystems that originate from distinct lineages and differentiate into a multitude of cellular subtypes or states. The spatial organisation of cells in tumours has been demonstrated to be not only a projection of the molecular nature of cancer but also an important predictors for the progression of the tumour and response to treatments. Conventional spatial technologies such as multiplexed immunofluorescence can only examine a handful of markers at a time, restricting our ability to comprehensively map the cellular and molecular features of tumours in tissue.

Several technologies have emerged to solve this challenge. Among these technologies, the GeoMX Digital Spatial Profiling (DSP) platform from Nanostring and the Visium platform from 10X Genomics have emerged as two powerful spatial transcriptomic tools with high data dimensionality and relatively high throughput. While both platforms have been demonstrated to generate robust outputs across a spectrum of tissues with high reproducibility, these two platforms vary substantially in experimental workflow and method of data collection. Previous comparisons of these two platforms are often based on data generated in unmatched samples without directly comparing technical features between the two platforms.

To bridge this gap, we evaluated the performance of the GeoMX DSP and Visium platforms on cultured cell line and clinical primary breast cancer patient samples. The former sample type consists of cell lines mixed at different ratios providing a well-controlled system for the direct technical comparison, while the latter extends our comparisons to a more practical setting. We applied the DSP and Visium platforms to matched fresh frozen and formalin-Fixed paraffin-embedded (FFPE) samples allowing a comprehensive comparison on all sample types supported by the two platforms. With the direct comparison between the DSP and Visium platforms, we provide a practical guide to prospective users of these technologies with technical comparisons, insights on experimental workflow and data processing to assist in decision-making when considering spatial transcriptomic experiments.

Dissecting the drivers of intra-tumoural heterogeneity in high grade brain tumours with single cell RNA-seq

Walter Muskovic[1], Ashraf Zaman[1], Madhavi Pandya[1], Emily Holton[1], Joseph E. Powell[1,2]

[1]Garvan-Weizmann Centre for Cellular Genomics, The Kinghorn Cancer Centre, Darlinghurst, New South Wales, Australia
[2]UNSW Cellular Genomics Futures Institute, University of New South Wales, Sydney, New South Wales, Australia

Intra- and inter-tumoural heterogeneity is one of the main drivers of treatment resistance in malignant brain tumours. Pre-existing tumour heterogeneity and acquired resistance enable tumour cells to survive chemotherapy and radiation treatment, facilitating the development of therapy-resistant tumour cell populations. Despite its clear clinical relevance, our understanding of the molecular mechanisms driving this heterogeneity remains fragmentary. To address this challenge, we dissociated tissue from 49 high grade brain tumour samples, comprising a mixture of primary and recurrent glioblastoma, oligodendroglioma and astrocytoma tumours. Using the gene expression profiles of 646,000 captured cells, non-negative matrix factorisation identified 11 malignant gene expression states. Comparison of these cell states to the lineage hierarchy of the developing brain revealed a close similarity to several normal brain cell types. Limited overlap between these cell states and the population of actively cycling tumour cells was observed. These results suggest the cell states may reflect aberrant developmental programs driven by a proliferating cancer stem cell population. To determine whether the direction in which malignant cells transition between states is consistent with the proposed malignant cell hierarchy a combination of RNA velocity and copy number variation analysis is being employed. By distinguishing between spliced and unspliced mRNA, RNA velocity can predict the future expression state of cells during dynamic processes such as differentiation. While powerful, this method is restricted to short time scales. To complement this approach, copy number changes are inferred using shifts in allele frequency and expression intensity across large segments of chromosomes. The evolutionary relationships between the uncovered subclones are used to determine the tumour clonal phylogeny, revealing the movement of cells between states over longer time scales. A more complete understanding of the drivers of tumour heterogeneity will advance our ability to develop targeted therapeutic approaches that prevent the emergence of resistance.

scFeatures: Multi-view representations of single-cell and spatial data for disease outcome prediction

Yue Cao[1,2], Yingxin Lin[1,2], Ellis Patrick[1,2,3], Pengyi Yang[1,2,3], Jean Yee Hwa Yang[1,2]

[1]Charles Perkins Centre, The University of Sydney, Sydney, Australia
[2]School of Mathematics and Statistics, The University of Sydney, Sydney, Australia
[3]Computational Systems Biology Group, Children's Medical Research Institute, Westmead, NSW, Australia

With the recent surge of large-cohort scale single cell research, it is of critical importance that analytical methods can fully utilize the comprehensive characterization of cellular systems that single cell technologies produce to provide insights into samples from individuals. Currently, there is little consensus on the best ways to compress information from the complex data structures of these technologies to summary statistics that represent each sample (e.g. individuals).

Here, we present scFeatures, an approach that creates interpretable cellular and molecular representations of single-cell and spatial data at the sample level. scFeatures generates features across six categories representing different molecular views of cellular characteristics. These include i) cell type proportions, ii) cell type specific gene expressions, iii) cell type specific pathway expressions, iv) cell type specific cell-cell interaction (CCI) scores, v) overall aggregated gene expressions and vi) spatial metrics.

Using multiple patient datasets, we demonstrate that summarising a broad collection of feature types at the sample level is both important for understanding underlying disease mechanisms in different studies and for accurately classifying disease status of individuals. We reveal that the combination of feature types, ie, the multi-view representation, achieves better classification performance compared to any of the individual feature types, therefore demonstrating the importance of generating a variety of feature types. Additionally, through a collection of COVID-19 datasets, we used scFeatures to uncover the different roles that comorbidity plays in disease severity in patients of different age groups.

Inferring cell diversity in single cell data using consortium-scale epigenetic data as a computational anchor for cell identity

Yuliangzi Sun[1,7], Woo Jun Shim[1,7], Sophie Shen[1], Enakshi Sinniah[1], Duy Pham[1], Zezhuo Su[2,3], Melanie D. White[1], Joshua W. K. Ho[4,5], Mikael Bodén[6], Quan Nguyen[1], Nathan J. Palpant[1]

[1] *Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia*
[2] *School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfuoam, Hong Kong SAR, China*
[3] *Laboratory of Data Discovery for Health Limited (D24H), Hong Kong Science Park, Hong Kong SAR, China*
[4] *Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Sydney, NSW, Australia*
[5] *University of New South Wales Cellular Genomics Futures Institute, Sydney, New South Wales, Australia*
[6] *School of Chemistry and Molecular Biology, The University of Queensland, Brisbane, QLD, Australia*
[7] *These authors contributed equally to this work*

Current clustering algorithms in single cell pipelines rely on mathematical principles to evaluate data complexity, and partitioning data without reference to biologically meaningful information. This study develops a cell type identification and visualisation method integrates epigenetic data from hundreds of diverse bio-samples as a biological reference point to infer cell diversity in single cell data. We use patterns of H3K27me3 domains deposited across >800 cell types to calculate a repressive tendency score (RTS), a quantitative value assigned to every gene that predicts cell-type specificity. Any orthologous single cell data can be analysed by this versatile genome-wide RTS values to identify cell-type specific genes. Using a topographical map concept, we utilize the rank order of genes based on RTS values as contour lines in a map to demarcate cell relationships. Using a weighted density estimation plot-based visualisation approach, RTS values act as a weighting parameter to determine cell populations in whole-genome UMAP space. Analysis using an adjusted rand index (ARI) demonstrates the requirement of RTS gene rank order in anchoring cell diversity across diverse in vivo and in vitro single cell data. Importantly, Spearman correlation analysis demonstrates significantly more diverse cell populations when anchoring single cell data using RTS values compared to equivalent clustering resolutions using methods such as Seurat. We couple this with an unsupervised machine learning method to evaluate orthogonal patterns of H3K27me3 depositions in hundreds of EpiMap cell types to efficiently group genes sharing similar biological processes from any single cell gene expression data. We demonstrate the power of these methods by analysing atlases of in vivo and in vitro cell organogenesis and diversification and provide a web accessible dashboard for access to data and software. Collectively, genome wide epigenetic repression provides a powerful biological reference point for identifying and studying genetic regulation in single cell expression data.

Identification of cell barcodes from long-read single cell RNAseq with BLAZE

Yupei You[1], Yair D.J. Prawer[2], Ricardo De Paoli-Iseppi[2], Cameron P.J. Hunt[3], Clare L. Parish[3], Heejung Shim[1] and Michael B. Clark[2]

[1]School of Mathematics and Statistics/Melbourne Integrative Genomics, The University of Melbourne, Parkville, VIC, 3010, Australia
[1]Centre for Stem Cell Systems, Department of Anatomy and Physiology, The University of Melbourne, Parkville, VIC, 3010, Australia
[3]The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC, 3010, Australia

Single cell RNA sequencing (scRNA-seq) has revolutionized our ability to profile gene expression. However, short-read (SR) scRNAseq methodologies such as 10x are restricted to sequencing the 3' or 5' ends of transcripts, providing accurate gene expression but little information on the RNA isoforms expressed in each cell. Newly developed long-read (LR) scRNA-seq enables the quantification of RNA isoforms in individual cells but largely relies on short-read sequencing to identify cell barcodes and allow single cell analysis. Here we introduce BLAZE (Barcode identification from long-reads for AnalyZing single cell gene Expression), which accurately and efficiently identifies 10x cell barcodes using only nanopore LR scRNA-seq data. We compared BLAZE to existing tools, including cell barcodes identified from matched SR scRNA-seq, on differentiating stem cells and 5 cancer cell lines. BLAZE outperforms existing tools and provides a more accurate representation of the cells present in LR scRNA-seq than using matched short-reads. BLAZE provides accurate cell barcodes over a wide range of experimental read depths and sequencing accuracies, while other methodologies commonly identify false-positive barcodes and cell clusters, disrupting biological interpretation of LR scRNA-seq results. In conclusion, BLAZE eliminates the requirement for matched SR scRNA-seq to interpret LR scRNA-seq, simplifying procedures and decreasing costs while also improving LR scRNA-seq results. BLAZE is compatible with downstream tools accepting a cell barcode whitelist file and is available at https://github.com/shimlab/BLAZE.

Identifying genetic features to predict and control cell differentiation lineages from pluripotency

Zhixuan Wu [1], Sophie Shen [1], Han Sheng Chiu [1], Nathan J. Palpant [1]

[1] *Institute for Molecular Bioscience, Brisbane, QLD, Australia*

Differentiation of human induced pluripotent stem cells (hiPSCs) into cardiomyocyte (CM) lineages are specified during the gastrulation-stage of development when precise timing and dosage of Wnt signalling controls derivation of mesoderm, endoderm, and ectoderm. We studied molecular mechanisms underlying dosage-dependent Wnt signalling during mesendoderm differentiation as a basis for identifying predictive gene markers and novel small molecules governing mesoderm differentiation into cardiomyocytes.

During the exit from pluripotency, hiPSCs were treated with increasing doses of Wnt agonist CHIR-99021 (1, 3, 5, 7 μM) and captured for single cell RNA sequencing at the gastrulation-stage of differentiation (day 2). Differential expression analysis between the CHIR-99021 dosages revealed 10 genes specifically and significantly upregulated in cardiogenic differentiation conditions (3uM CHIR-99021). To test whether these genes are predictive of cardiac lineage differentiation, we evaluated the relationship between gene expression and cardiac differentiation using two independent iPSC lines differentiated under diverse seeding densities and CHIR-99021 concentrations. Results show that expression of genes associated with retinoic acid and mesendoderm at day 2 are significantly positively correlated with cardiomyocyte differentiation efficiency at day 10.

We queried lineage-specific gene expression profiles with the Connectivity Map database to identify novel small molecules predicted to promote specific cell differentiation trajectories. Among three candidates, we identified the antiallergic drug as a novel small molecule associated with cardiac differentiation and demonstrate its ability for to rescue cardiac differentiation under sub-optimal Wnt stimulation (1 μM CHIR-99021). We aim to test its ability to rescue cell differentiation in hiPSCs with low endogenous Wnt signalling and assess its mechanisms and functionality during hiPSCs-CMs differentiation.

Collectively, this study provides insights into the relationship between gene expression programs, cardiac differentiation efficiency, and Wnt-signalling dosage to enable the selection of iPSCs with high cardiac differentiation potential to facilitate time and cost-effective generation of cardiomyocytes.

Modeling group heteroscedasticity for pseudo-bulk samples in single-cell RNA-seq data

Yue You[1], Xueyi Dong[1], Peter Hickey[1], Matthew Ritchie[1], Gordon Smyth[1], Charity Law[1]

[1]Walter and Eliza Hall Institute of Medical research

Group heteroscedaticity is commonly observed on pseudo-bulk single cell RNA-seq datasets, which raises an issue in the search for differentially expressed genes. Most bulk RNA-seq methods assume equal group variances meaning that group variances can be under- and/or over- estimated. We provided two methods that account for heteroscedastic groups, namely voomByGroup and voomWithQualityWeights using a blocked design (voomQWB). As compared to current gold-standard methods that do not account for heteroscedasticity, our simulation studies and case studies demonstrate the superior performance of both voomByGroup and voomQWB in error control and power when group variances of pseudo-bulk scRNA-seq data are unequal. We recommend the use of either of these methods over these gold-standard methods, with voomByGroup having the advantage of accurate variance estimation since group variance trends can take on different “shapes”, whilst voomQWB has the advantage of catering to complex study designs.

Interrogating immune cell heterogeneity of human small cell lung cancer

J. Ng[1,2], Y. You[1], N. Gherardin[2], J. Hess[1], A. Kersbergen[3], D. Godfrey[2], S. Best[1,2], T. Leong[1,3], M. Ritchie[1,2], P. Hickey[1,2], D. Steinfort[2,5] & K. D. Sutherland[1,2]

[1]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.
[2]Department of Medicine, Faculty of Medicine, Dentistry & Health Sciences, University of Melbourne, Australia.
[3]Peter MacCallum Cancer Centre, Melbourne, Australia.
[4]Austin Health, Melbourne, Australia.
[5]Royal Melbourne Hospital, Department of Respiratory Medicine, Melbourne, Australia.

Small cell lung cancer (SCLC) is one of the deadliest cancers with an extremely low five-year survival rate of <6%. First-line treatment with platinum-based chemotherapy is ineffective and almost always results in rapid relapse. Moreover, immune checkpoint blockade (ICB) together with chemotherapy only marginally increased the survival of SCLC patients. Prior studies of SCLC patient material have revealed an immunosuppressed microenvironment that may contribute towards ineffective ICB. As such, a deeper understanding of the landscape of infiltrating immune cells in SCLC is needed to identify alternative immunotherapeutic strategies to increase the survival of SCLC patients.

To interrogate the immune cell heterogeneity of SCLC, we performed single cell RNA-sequencing using CEL-Seq 2.0 on SCLC patient fine needle biopsy aspirations (n=11) of metastatic tumours from thoracic lymph nodes (LN) and one lung tumour resection. CEL-Seq 2.0 allows us to capture and enrich for immune cell populations at a deeper sequencing depth.

Interestingly, γδ T cells were found to be enriched in SCLC-affected LN samples, compared to patient-matched unaffected LN biopsy samples. γδ T cells play a critical role in immune surveillance of malignant cells and can exhibit anti-cancer functions. Importantly, the role of γδ T cells in SCLC tumour progression is still unknown. We used a high-parametric flow cytometry panel to characterise the phenotype of γδ T cells (Vδ2+ or Vδ2-), including features of residency, activation status and checkpoint expression. Current investigations are focused on understanding the T cell receptor repertoire of invariant Vδ2- T cells to assess their specificity in recognising tumour-specific antigens and evaluating whether γδ T cells serve as a prognostic marker in SCLC patients. Overall, these findings aim to provide a new path forward for SCLC immunotherapy through the modulation of γδ T cells.

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Time course analysis of pseudo-bulk samples from single cell RNA sequencing data

Jinming Cheng[1,2], Yunshun Chen[1,2,3], Gordon K. Smyth[1,4]

[1]Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia
[2]Department of Medical Biology, The University of Melbourne, Parkville, VIC 3010, Australia
[3]ACRF Cancer Biology and Stem Cells Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia
[4]School of Mathematics and Statistics, The University of Melbourne, Parkville, VIC 3010, Australia

Single cell RNA sequencing technologies have been rapidly developed in recent years. The 10x droplet-based single cell RNA sequencing technology makes it possible to profile gene expression of tens of thousands of cells per sample. Standard analysis of single cell RNA sequencing data usually includes quality control, normalization, dimension reduction, cell clustering and differential expression analysis. Removing the potential doublets is also recommended in the the standard analysis. Multiple samples at different stages can be integrated together, and the downstream trajectory analysis can be performed to study the cell development process. Here, we further extend the downstream analysis to time course analysis taking advantage of the pseudotime inferred from trajectory analysis. In this workflow, we use single cell RNA sequencing data of mouse mammary gland epithelium at five different stages to demonstrate the standard analysis and integration analysis with Seurat, the doublet prediction with scDblFinder, the ternary plot analysis using signature genes, the trajectory analysis with monocle3, and time course analysis using pseudo-bulk data with edgeR.

Comparing single cell datasets using DensityMorph

Kristen Feher[1]

[1]*Bioinformatics Support Facility, WEHI*

The proliferation of single cell datasets has brought a wealth of information, but also great challenges in data analysis. Obtaining a cohesive overview of multiple single cell samples is difficult and requires consideration of cell population structure - which may or may not be well defined - along with subtle shifts in expression within cell populations across samples, and changes in population frequency across samples. Ideally, all this would be integrated with the experimental design, e.g. time point, genotype, treatment etc. Data visualisation is the most effective way of communicating analysis but often this takes the form of a plethora of TSNE plots, colour coded according to marker and sample. In this manuscript, I introduce a novel exploratory data analysis and visualisation method that is centred around a novel quasi-distance (DensityMorph) between single cell samples. DensityMorph makes it possible to plot single cell samples in a manner analogous to performing principal component analysis on microarray samples. Biological interpretation is ensured by the introduction of Explanatory Components, which show how marker expression and coexpression drive the differences between samples. This method is a breakthrough in terms of displaying the most pertinent biological changes across single cell samples in a compact plot. Finally, it can be used either as a stand-alone method or to structure other types of analysis such as manual flow cytometry gating or cell population clustering. The method is demonstrated on a COVID flow cytometry dataset stained with a leukocyte panel.

Investigating the influence of X chromosome inactivation on the female bias in autoimmune disease

Lachlan G Gray[1,2], Joseph Powell[1,3], Sara Ballouz[1,2]

[1]*Garvan Institute of Medical Research, Sydney, Australia*
[2]*Faculty of Medicine, University of New South Wales, Sydney, Australia*
[3]*Cellular Genomics Futures Institute, University of New South Wales, Sydney, Australia*

To account for two copies of the X chromosome, females randomly inactivate one chromosome to maintain gene dosage between the sexes. However, 15-23% of genes on the inactivated chromosome can escape this silencing. It has been proposed that these escape genes promote immune responses towards autoreactivity and may contribute towards the female bias in autoimmune disease, where 80% of patients are female.

In this study, we have analysed public single-cell RNA sequencing data from females with Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS), primary Sjogrens Syndrome (pSS) and Ulcerative Colitis (UC) to reveal cell-specific expression of genes escaping X chromosome inactivation (XCI). Utilising the edgeR-likelihood ratio test pseudobulking approach, we performed a cell-type specific differential expression analysis. To measure a joint autoimmune signature, we integrated these five datasets (across 125,894 cells and 24 cell types) and repeated our analysis.

With the integrated analysis, on average 1,294 genes were differentially expressed per cell-type, with 11 escaping XCI (FDR < 0.05, |logFC| > 0.5). Notably, biglycan (BGN) with a role in SLE, was upregulated in disease cohorts across multiple cell types. Of the differentially expressed escape genes, many play key immunological roles including antimicrobial, cytokine, chemokine, T cell receptor and B cell receptor signalling pathways. Using disease specific genes to train a randomForest model, we classified patients within the joint dataset (holding out those used for training). We find that differentially expressed genes, including escape genes, from B intermediate, erythrocytes and plasmablast cells accurately classified individuals into cases and controls (AUC > 0.8).

Our results suggest that the disease-specific differentially expressed genes are good markers for a broad immune dysfunctional signature. As XCI occurs only in females, the subset that escape XCI support a potential role for their contribution in the sexually dimorphic nature of autoimmune disease.

Myeloid-derived suppressor cells regulate metastatic dissemination in triple-negative breast cancer

Laura Rodriguez de la Fuente[1,2,3], Yolanda Colino-Sanguino[3,4], Jeron Venhuizen[1], Andrew MK. Law[1,2], Fatima Valdes-Mora[3,4] and David Gallego-Ortega[1,2,5]

[1]Tumour Development Laboratory, The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, NSW Australia
[2]St. Vincent's Clinical School, Faculty of Medicine, UNSW Sydney, NSW, Australia
[3]Cancer Epigenetic Biology and Therapeutics. Precision Medicine Theme. Children's Cancer Institute, Lowy Cancer Research Centre, UNSW Sydney, Sydney, NSW, Australia Sydney, NSW Australia
[4]School of Women's and Children's Health, Faculty of Medicine, UNSW Sydney, NSW Australia [5]Centre for Single Cell Technology. School of Biomedical Engineering, Faculty of Engineering and IT. University of Technology Sydney, Ultimo, NSW, 2007 Australia.

The prognosis for patients with triple-negative breast cancer (TNBC) remains poor, with newly developed immunotherapies failing to produce the benefits seen in other cancers. This failure is often associated with the presence of myeloid-derived suppressor cells (MDSCs): an immune population with high plasticity and potent immunosuppressive capacity that plays an active role in the development of metastasis, the primary cause of death in TNBC patients. Targeting MDSCs is an attractive therapeutic strategy, however the specific molecular features that define their identity, and the inflammatory mechanisms these cells employ to drive metastatic spread, remain elusive.

Using a single-cell multi-omic approach, we profiled the tumour microenvironment and lung cellular ecosystem before and after metastatic colonization in the highly metastatic 4T1.2 model of TNBC. Comparatively, we used the non-metastatic 67NR model as a baseline to identify the pro-metastatic MDSCs and define the specific mechanisms that contribute to cancer dissemination.

We have created a multi-omic spatiotemporal atlas of inflammation associated with breast cancer metastasis in TNBC. Our scRNAseq approach revealed the composition and functional diversity of inflammatory cells in tumours and metastatic sites, identifying a subclass of granulocytic MDSCs involved in the formation of the pro-metastatic niche and progression to metastatic disease. We further characterized this pro-metastatic MDSC subclass integrating the scRNAseq with chromatin accessibility (scATACseq) and a high-dimensional (100+) cell surface antibody panel (CITE-seq) to reveal molecular targets with potential therapeutic value. Our multi-omic definition of pro-metastatic MDSCs is a step forward for designing new strategies to efficiently reprogram immunosuppressive mechanisms and stimulate antitumor immunity, ultimately paving the way for the development of the next generation of immunotherapy for metastatic breast cancer.

Profiling Cellular and Molecular Mechanisms of Chronic Pain with Integrated Transcriptomics

Lipin Loo [1]

[1] *Charles Perkins Centre, University of Sydney*

Neuropathic pain, pain caused by damage or disease affecting the somatosensory nervous system (e.g., sciatica, back pain, cancer pain, diabetic pain, accidental injury) is particularly refractory to available therapies, with front line anti-neuropathic drugs providing adequate pain relief for only around 25% of patients. These drugs inhibit hyperactive pain signals but also inadvertently affect other neurons in the nervous system, making them unsuitable for long-term use. To effectively treat chronic pain, we require better targeted therapies to control hyperexcitability in the neuropathic spinal cord without affecting the entire nervous system. To this end, we have developed a protocol for a cell therapy that boosts inhibition locally in the spinal cord and this single spinal injection of “pain killer” inhibitory neurons (derived from human induced pluripotent stem cells; iPSC) can provide ~permanent neuropathic pain relief in mice. Our strategy to comprehensively understand neuropathic pain is by firstly reconstructing and comparing the systems involved in pain signalling under normal and neuropathic conditions. Here, we have reconstructed the healthy and neuropathic spinal cord using single nucleus RNAseq (snRNAseq), a technique which avoids cell preparation biases and enriches for nascent transcripts representative of the immediate state of the neurons at the time of dissociation, and spatial transcriptomics, a technique that maps whole transcriptome data with anatomical context. Furthermore, by characterizing the neuropathic spinal cords that received “pain killer” injections, we identified neuropathic signalling pathways that were reversed for pain relief. Interestingly, our single cell analysis of the neuropathic spinal cord reveals intricate changes in only a subpopulation of cells while transplanted “pain killer” cells exerted widespread dampening of transcriptional activity. Our findings here will instruct our future work in stem cell differentiation and CRISPR-mediated gene editing, to generate precise therapies that target the cellular and molecular drivers of chronic pain.

Multi-omic characterization of the response to immunotherapy in murine breast cancer models

Louise Baldwin[1,2], Jessica Yang[2], Kate Harvey[2], Ghamdan Al-Eryani[1,2], Simon Junankar[1,2], Kylie James[1,2], Alexander Swarbrick[1,2]

[1]The Kinghorn Cancer Centre, Garvan Institute of Medical Research, NSW 2010, Australia
[2]School of Clinical Medicine, Faculty of Medicine UNSW Sydney, NSW 2010, Australia

Immune checkpoint inhibitors (anti-CTLA-4 and anti-PD-1/L1), or immunotherapy, have been a breakthrough treatment for various malignancies. Breast cancer is relatively immune evasive and responds poorly to immune checkpoint inhibition. Cellular multi-omic technologies are well placed to shed light on how immunotherapy shapes cancer cells, the tumour microenvironment and the immune landscape concurrently. Existing research in this space has largely focused on patient tissue, but clinical samples are limited in terms of sample size, collection of matching relevant tissues and experimental manipulation of the system.

Here we apply single cell multi-omics (transcriptomics and antigen receptor profiling) to two murine models of breast cancer. The 4T1 (non-responsive) and EMT6 (responsive) models were generated on the BALB/c background, eliminating host genetic variability. By collecting tumour and matched draining lymph nodes at baseline and midway through immunotherapy treatment, we can take a deep dive into the immunotherapy response as it occurs. Data from EMT6 and 4T1 model is has been generated and the analysis is to begin shortly.

This research enables us to examine what facilitates or prohibits a successful anti-cancer immune response in the context of immunotherapy. By parsing any resulting candidate genes or pathways over human cancer single cell data, we aim to identify clinically relevant targets to improve patient response to immunotherapy. Additionally, we also hope to shed light on the debated mechanistic differences between combination and single agent immunotherapy. Finally, we will carry out a comprehensive comparative analysis of mouse and human immunology. In conclusion, this research will contribute valuable mechanistic knowledge to our understanding of immunotherapy, the murine immune system and response to immunotherapy in a tightly controlled experimental setting.

TrajectoryNet reveals temporal transcriptional relationships driving the mesenchymal-to-epithelial transition in tumour formation

Shabarni Gupta[1], Alexander Tong*[2], Manik Kuchroo*[3], Beatriz P. San Juan[1], Laura Rangel[1], Brandon Zhu[4], John G. Lock[5], Christine L. Chaffer[§1,6], Smita Krishnaswamy[§2,7]

[1] *The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Darlinghurst 2010, Australia*
[2] *Department of Computer Science, Yale University, New Haven, CT, USA*
[3] *Department of Neuroscience, Yale University, New Haven, CT*
[4] *Yale College, New Haven, CT, USA*
[5] *University of New South Wales, Kensington, New South Wales, Australia*
[6] *St Vincent's Clinical School, University of New South Wales Medicine, University of New South Wales, Darlinghurst 2010, Australia*
[7] *Department of Genetics, Yale University, New Haven, CT, USA*
** First authors, [§] Corresponding authors*

Background: Metastasis is mediated through cancer stem cells (CSCs) with tumour-initiating properties that create heterogeneity critical to robust tumour growth. The latter is achieved through their ability to differentiate into non-CSC progeny. These dynamic cell-state transitions are regulated through intrinsic programs such as the epithelial-to-mesenchymal transition (EMT), driving non-CSC to CSC de-differentiation, and the reverse mesenchymal-to-epithelial transition (MET) driving CSC to non-CSC differentiation.

Aim: This study aims to define the dynamic transcriptomic transitions that drive CSC into a chemotherapy-sensitive non-CSC state through the MET program.

Methods: We developed a neural ODE network called TrajectoryNet that learns continuous dynamics from static transcriptomic data and applied it to time lapsed single cell expression measurements from an in-vitro triple negative breast cancer MET differentiation system. This led to the identification of 23 core transcriptional factors that were associated with the emergence of the epithelial trajectory (non-CSC state). We, next, constructed a gene regulatory network to sketch the transcriptional circuitry underlying the MET. The regulatory effect of one such gene, the estrogen related receptor alpha (ESRRA) was validated using orthogonal approaches including RNAi, western blotting and immunofluorescence.

Results: Using TrajectoryNet we mapped the first temporal gene expression program driving the MET. Using the core transcription factors identified through TrajectoryNet we identified a sub-network initiating MET through early elevated expression of ESRRA. Indeed, knockdown of ESRRA increased CDH1 (E-cadherin) expression. The downregulation of ESRRA within tumorsphere was validated temporally, together with the concomitant downregulation of ZEB1 and upregulation of CDH1.

Conclusions: TrajectoryNet presents an innovative approach to characterizing the dynamic molecular programs derived from time-point based transcriptomic datasets. Using this approach, we define the key transcription factors driving the MET that have broad implications for translational research aimed at driving CSCs out of their aggressive state as a means to inhibit metastasis and chemotherapy-resistant disease.

Deconstructing epithelial ovarian cancers using single cell gene expression data

Masih Sherafatian[1], Suad Abdirahman[1], Luciano Martelotto[2], Heather Thorne[3], Fernando Rossello[2], Matthew Wakefield[4], Kylie Gorringe[1]

[1]*Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC, Australia*
[2]*Department of Clinical Pathology and Centre for Cancer Research, University of Melbourne, Melbourne, VIC, Australia*
[3]*Cancer Genomics Program, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia*
[4]*The Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia*

The tumour mass of epithelial ovarian cancers consist of a heterogenous population of malignant and supporting cells. Correctly identifying and characterizing these cells is fundamental to understanding tumour evolution and heterogeneity. Currently, a few universal expression markers are widely used to classify its components. However, analysis of the epithelial tumours at single cell resolution can better characterize these components in different types of epithelial cancers and infer their lineage.

In order to deconstruct epithelial ovarian cancers, we performed single RNA-seq on ovarian and fallopian tube tissues of a BRCA1 carrier individual and characterized cell types from these tissues. Expression profiles from the annotated cell types were used as references to deconvolute the components of different serous ovarian cancers. Moreover, inferred copy number variation (CNV) and Transcription Factor (TF) analysis were performed to validate their lineage.

We discovered small populations of novel PAX8-negative epithelial cell types with stem cell-like features in both the FT and ovary of the BRCA1 individual. We hypothesised that these cells could be a potential cell-of-origin for ovarian cancer. Deconstruction of the tumour data in multiple published serous ovarian carcinomas revealed the presence of similar PAX8- cell populations in different tumour subtypes. Surprisingly, these cells carried both mesenchymal and epithelial markers, and, inferred CNV analysis and TF analysis suggested a shared tumour epithelial lineage as PAX8+ cancer cells. We hypothesise that these cells could be “leader” cells and “cancer stem cells” with characteristics enabling metastatic spread and stemness.

We discovered novel potential cells of origin from FT and OSE of a BRCA1 mutation carrier. We also characterized cancer cell clusters and their contribution in different serous subtypes. Our findings shed new light on leader cell and cancer stem cell populations in ovarian serous tumours and could lead to early disease biomarkers for ovarian cancer and its metastasis.

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Comparison of 10x Genomics 3'v3.1 and ParseBio Evercode technologies on myoblast nuclei

Matthew E Jones [1], Irina Kuznetsova [1], Caleb Thomas [1], Khaing Hmon [1], Gina Ravenscroft [1], Sue Fletcher [2], Elena Denisenko [1], Alistair R R Forrest [1]

[1] Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, The University of Western Australia, Nedlands, Perth, WA 6009, Australia
[2] Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University WA 6150 and PYC Therapeutics, Nedlands, WA 6009

The vast majority of single cell profiling worldwide has to date used the highly successful 10x genomics microfluidic kits and controllers. Although robust and allowing rapid uptake of single cell profiling, the costs of experiments are still a barrier to entry for many labs and limit our ability to carry out studies on clinical samples of sufficient size and power. Recently, multiple new alternative single cell profiling technologies have become commercially available that promise to substantially reduce the cost per sample. This poster builds on the technology benchmarking our lab has performed, presented by Irina Kuznetsova, by showing companion data on myoblast nuclei processed with 10x Genomics 3'v3.1 and ParseBio Evercode kits.

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Disparities in spatially variable gene calling highlight the need for benchmarking methods

Natalie Charitakis [1,2,4], Kevin I. Watt [1,4,5], Enzo Porrello* [1,3,4], David Elliott* [1,2,4], Mirana Ramialison* [1,2,4]

[1] *Murdoch Children's Research Institute, Royal Children's Hospital, Flemington Road, Parkville, Victoria, 3052, Australia,*
[2] *Department of Paediatrics, University of Melbourne, Grattan Street, Parkville, Victoria, 3010, Australia,*
[3] *Department of Anatomy and Physiology, University of Melbourne, Grattan Street, Parkville, Victoria, 3010, Australia,*
[4] *Novo Nordisk Foundation Center for Stem Cell Medicine, Murdoch Children's Research Institute, Royal Children's Hospital, Flemington Road, Parkville, Victoria, 3052, Australia.*
[5] *Department of Diabetes, Monash University, Alfred Centre, Commercial Road, Melbourne, Victoria, 3004, Australia*

Introduction: Spatially resolved transcriptomics (SRT) is a novel, disruptive technology set to push the boundaries of exploring gene regulatory networks while providing both spatial and temporal resolution. It is expected to be exponentially adopted by the transcriptomics community after being named Nature's Method of the Year in 2020. Despite this, analysis packages for SRT datasets are still in their infancy, with a clear forerunner yet to emerge. A comprehensive overview of the performance of commonly used packages on the same datasets is lacking and there is a need to determine their performance in correctly labelling spatially variable genes (SVGs).

Methods : To establish which of the current packages is most effective in identifying SVGs within datasets generated using the same technology, a combination of publicly available and simulated 10X Genomics Visium datasets generated from 8 fresh frozen and FFPE human tissue samples were analysed. I will be assessing the performance of SpatialDE, SPARK, SpaGCN, scGCO and Seurat as each uses a different mathematical model to identify SVGs. Separate simulated datasets with known true positives or randomised signal helps begin to elucidate package performance.

Results: In all datasets, most packages identify SVGs independent of results from other packages and overlap is minimal. The number of SVGs identified by each package differ substantially and differences are statistically significant. SpaGCN appears consistently the most conservative while SpatialDE consistently labels the greatest number of genes as SVGs. Many datasets have an underlying distribution of gene expression that does not fit the assumptions of many of the packages, which may be affecting performance.

Conclusion: Given the disparity of results, this comparison study highlights the need of further development of benchmarking methods for identifying SVGs in SRT experiments. This should include methods for reliably simulating SRT data for method validation.

Streamlining end-to-end CyTOF data analysis

Niko Thio[1], Sean MacDonald[1], Criselle D'Souza[1], Paul Neeson[1]

[1]Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia

Cytometry by time of flight (CyTOF) is a platform for high-dimensional phenotypic and functional analysis of single cell level. The system uses monoclonal antibodies conjugated to metal isotopes to evaluate a panel of 40+ parameters (with theoretical upper limit of 120 parameters) on single cell with minimal channel overlap. The panel can be customised for both phenotypic and functional markers. Compared to scRNAseq, CyTOF allows data acquisition in the range of 106-107cells per sample, which facilitates characterisation of rare cell population and also provides functional information through protein analysis.

CyTOF data analysis involves multiple stages of processing, such as pre-processing (Quality Control, normalisation, filtering, batch consolidation), cell phenotyping (clustering, annotation), and downstream analysis (abundance analysis and visualisation). On another aspect, end-to-end analysis involves people with multidisciplinary roles, for example: principal investigator, laboratory scientist, and bioinformatics analyst. Each role contributes unique expertise and information into the processing stages, resulting the overall analysis as an iterative process rather than a simple linear pipeline.

In this presentation, we describe our current workflow for streamlining CyTOF end-to-end data analysis process, with aim to accommodate the iterative nature of the process. We start by describing our early iteration of the workflow on selected projects (tumour immune microenvironment profiling in vaginal melanoma study, and BIA-ALCL study), and then presenting the revised processing workflow that aimed for larger scale and involving multiple batches.

The main highlights of this workflow are firstly, an automated phenotyping using staged clustering/gating strategy that allows manual refinement, and secondly, a framework that allows (re)applying revisions and version management to support rapid iterations.

Population scale stem cell biology with induced pluripotent stem cells during cardiac differentiation

Nona Farbehi[1], Angela Steinmann[1], Himanshi Aurora[1], Drew Neavin[1], Anne Senabouth[1], Vikkitharan Gnanasambandapillai[1], Chialing Chan[1], Eric Lam[1], Karena Pryce[1], Alex W. Hewitt[1,5,6], Alice Pebay[3,4], Joseph Powell[1,2]

[1]Garvan Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Sydney, NSW 2010, Australia
[2]UNSW Cellular Genomics Futures Institute, University of New South Wales, Sydney, NSW, 2052, Australia
[3]Department of Anatomy and Physiology, The University of Melbourne, Parkville, VIC 3010, Australia
[4]Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, East Melbourne, VIC 3002, Australia,
[5]Wicking Dementia Research and Education Centre, University of Tasmania, Hobart, Australia
[6]School of Medicine, Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia

Human induced pluripotent stem cells (hiPSCs) are a renewable resource enabling the investigation of genetic regulation of molecular phenotypes, and the role of these effects on human disease. hiPSCs can be used to identify how common DNA variation shapes cellular phenotypes. However, efforts to ascertain effects of human genetic variation on cellular phenotypes encounter major challenges which involve reaching the required scale by culturing the large number of lines necessary to associate phenotype with genotype. Our approach to solving these challenges is through the use of hiPSC 'village' experimental design, which involves the culture and differentiation of multiple hiPSC lines in a single dish. This experimental framework provides the statistical power to link genetic variation to cell phenotypes and reduces technical variation due to cell culture effects. By differentiating hiPSCs down the mesoderm lineage and using single nucleus Multiome sequencing, we study genetic effects on variation of gene regulation and cell fate as cells move across a developmental lineage. We have generated Multiome data from ~376,000 nuclei at eight time points across cardiac differentiation lineages and map cell-type specific genetic effects on gene expression (eQTLs) and chromatin accessibility (caQTLs). Cells were clustered and classified using Harmony and ArchR. Individual lineage developmental trajectory is compared using a combination of RNA-velocity (within time-points) and Slingshot (between time-points). We demonstrate that population-scale hiPSC models can be used to uncover genetic control of cell phenotypes in a cost-effective manner. This work shows multi-faceted evidence of molecular mechanisms leading to variation in cell behavior.

Flexiplex: A Flexible Demultiplexer and Search Tool for Single Cell Data

Noorul Amin[1], Nadia Davidson[1]

[1]Walter and Eliza Hall Institute of Medical Research

In sequencing data analysis, searching for and extracting a target sequence from reads has become a routine task. For instance, assigning single cell reads to cellular barcodes and UMIs, or genotypes to cells. However, the existing tools which perform this function are often task-specific (i.e only demultiplex barcodes for particular types of experiments) or are not tolerant to noise in the sequencing data. To overcome these limitations, here we present Flexiplex, a versatile and lightweight sequence searching and demultiplexing tool for omics data. We will show that Flexiplex can be used to extract cellular barcodes corresponding to specific alleles, splicing, or fusion events in both long and short read single cell RNA-seq. Moreover, Flexiplex works in two modes; a) when the target sequence is known and b) discovery mode – when only flanking sequencing of a region of interest is known. Flexiplex is based on Levenshtein distance and thus allows error-tolerant matches which yield robust searches. Flexiplex is available at https://davidsongroup.github.io/flexiplex/.

STimage: deep learning inferences of cancer gene and cell markers using histological tissue images and spatial transcriptomics data

Xiao Tan [1], Onkar Mulay [1], Samual MacDonald [2], Maciej Trzaskowski [2], Quan Nguyen* [1]

[1] Genomics and Machine Learning Lab, Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia
[2] Max Kelsen company, Brisbane, Australia

Spatial transcriptomics (ST) technology can measure most of the possible genes present in a tissue section, preserving the spatial location and tissue morphology information. Such multi-modal data provides more comprehensive information about the visible (tissue image) and invisible features (molecular profiles) from the same tissue section. Deep learning methods for cancer imaging data have been well developed, opening a vast potential for computer-aided tools for cancer diagnosis. However, such methods lack the ability to link biological processes/mechanisms to computational predictions, and thus having low interpretability.

Here, we developed STimage, an innovative approach to integrate both imaging and sequencing data to solve the limitations of current machine learning methods by predicting gene expression using histological images. STimage implements a deep learning framework combined with a statistical approach to build regression models to estimate the negative binomial (NB) distribution and predict real expression values of gene markers or a group of genes of cancer tissues. Utilising unique features of ST data, where both H&E images and gene expression data are measured, we gain power and resolution for training the regression models. We demonstrated the model applicability in predicting breast cancer biomarkers in independent ST and H&E image from TCIA (non-ST dataset) using our trained model. This allows for broader and more cost-effective approaches as the input data of a trained model is just a H&E image as used in a standard pathological workflow. We also implemented interpretability analysis, which use LIME model to investigate how model learn the differences in immune and cancer cells, and uncertainty analysis about variance measurement for the NB prediction to assess the confidence and reliability of the models.

STimage contributes to the integration of histological imaging and sequencing data to generate insights into tumour heterogeneity and has potential for cancer diagnosis in clinical practice.

Temporal transcriptomic dynamics of the ATX-LPAR-PLPP axis during skeletal muscle regeneration at single cell resolution

Osvaldo Contreras[1,2]

[1]Developmental and Regenerative Biology Division, Victor Chang Cardiac Research Institute
[2]Faculty of Medicine and Health, UNSW Sydney, Australia

Lysophosphatidic acid (LPA) is a growth factor-like bioactive phospholipid. As such, it has recently become an attractive target during tissue inflammation, repair, and regeneration, demonstrating biomedical relevance. LPA and LPA receptor-mediated signalling pathways regulate embryonic development, wound healing, carcinogenesis, and fibrosis, inducing cell migration, proliferation, and differentiation. Extracellular LPA is produced by the secreted hydrolase ectonucleotide pyrophosphatase/phosphodiesterase 2 (Enpp2, Autotaxin/Atx) and hydrolyzed by different membrane-bound phospholipid phosphatases (PLPPs). This study uses bulk and single-cell RNA sequencing to explore the gene expression dynamics of LPA receptors (Lpa1-6), Enpp2, and Plpp in skeletal muscle cells in homeostatic conditions and regeneration. Here, I show that skeletal muscle differentially expresses Lpar-Enpp2/Atx-Plpp coding genes, being Lpar1 the highest expressed member among LPARs. Lpar1 was highly expressed by FAPs and tenocytes, whereas Enpp2/Atx was mainly by FAPs. Clustering stromal fibro-adipogenic progenitors (FAPs) identified different populations representing distinct cell states with robust Lpar and Enpp2/Atx transcriptome signatures in homeostasis or resting-like cell states, including Dpp4+ and Hsd11b1+ FAPs. Tissue injury induces strong and fast repression of LPA receptors and Enpp2/Atx, indicating activated and proliferative FAP cell states are partly defined by a strong downregulation of LPAR and Enpp2/Atx gene expression. Hence, our results highlight the presence of the Atx-Lpar-Plpp axis in different muscle cells and FAP lineage in homeostasis and injury, providing a robust entry point for profound research on the role of LPA signalling skeletal muscle and other organs and tissues.

Investigation of metastatic-castration resistant prostate cancer heterogeneity using single-cell technologies

Raisa Hasan [1,2], Kate Harvey [2], Eva Apostolov [1,2], Priyanka DuttaGupta [3], Kiranj Chaudagar [3], Nenad Bartonicek [1,2], Ghamdan Al Eryani [1,2], Akash Patnaik [3], Alex Swarbrick [1,2]

[1] Cancer Ecosystems Program, Garvan Institute of Medical Research, NSW 2010, Australia
[2] St. Vincent's Clinical School, Faculty of Medicine UNSW Sydney, NSW 2010, Australia
[3] Hematology/Oncology, Department of Medicine, University of Chicago, Chicago 60637, IL, US

Metastatic castration-resistant prostate cancer (mCRPC) is a lethal and clinically challenging to diagnose form of advanced prostate cancer (PCa). Patients that exhibit mCRPC pathogenesis experience progressive levels of resistance to many forms of mainstay treatments such as chemotherapy, surgical procedures and hormone-targeting inhibitors of the androgen-receptor (AR) pathway. This is attributed to mCRPC extensive cellular and molecular heterogeneity, which current histopathological features used in the clinic in the diagnosis of PCa inadequately represents.

Currently, the confronting challenge of mCRPC is the stratification of this heterogeneous disease into molecularly and clinically informed groups, and the identification of biomarkers that predict their genesis during initial manifestation and treatment of PCa. In a collaboration with the Patnaik Lab at the University of Chicago, we generated a unique single-cell cohort of ~7,000 cells from five mCRPC patients.

The understanding of mCRPC at the cellular resolution can initiate the investigation of the major cell compartments involved in driving mCRPC and its' resistance to mainstay treatments. Specifically, our work is focused on looking at the interactions between the epithelial and T-cell compartments. The characterisation of both these cellular compartments in mCRPC is an extreme challenge due to lack of access to metastatic lesions, and difficulties in processing tissues for quality single-cell sequencing.

Our lab has been able to sequence thousands of epithelial cells, exhibiting inter-patient heterogeneity through the appearance of distinct patient-specific clustering and the heterogenous expression of key molecular drivers, such as AR. In conjunction, the performance of copy number variation analysis will allow us to further classify the heterogeneity of these cancer cells. Furthermore, mCRPC T-cell response is inadequately investigated, specifically antigen elicited drivers for immunity which can highlight a potential avenue for immunotherapy. This can be done through clonality analysis from single-cell VDJ receptor sequencing which our lab has also conducted on our cohort.

Personalised haplotype assembly and comparative crossover map analysis using single-gamete sequencing data

Ruqian Lyu[1,2], Vanessa Tsui[3,4], Wayne Crismani[3,4], Ruijie Liu[1], Heejung Shim[2] and Davis J. McCarthy[1,2]

[1]*Bioinformatics and Cellular Genomics St. Vincent's Institute of Medical Research*
[2]*Melbourne Integrative Genomics, Faculty of Science, The University of Melbourne*
[3]*DNA Repair and Recombination Laboratory, St Vincent's Institute of Medical Research*
[4]*The Faculty of Medicine, Dentistry and Health Science, The University of Melbourne*

Profiling gametes of an individual enables the construction of personalised haplotypes and meiotic crossover landscapes, now achievable at larger scale than ever through the availability of high-throughput single-cell sequencing technologies. However, high-throughput single-gamete data commonly have low depth of coverage per gamete, which challenges existing gamete-based haplotype phasing methods. In addition, haplotyping a large number of single gametes from high-throughput single-cell DNA sequencing data and constructing meiotic crossover profiles using existing methods requires intensive processing. Here, we introduce efficient software tools for the essential tasks of generating personalised haplotypes and calling crossovers in gametes from single-gamete DNA sequencing data (sgcocaller), and constructing, visualising, and comparing individualised crossover landscapes from single gametes (comapr). Using public datasets, we demonstrate that sgcocaller can generate impeccable phasing results for high-coverage datasets, on which it is more accurate and stable than existing methods, and performs well on low-coverage single-gamete sequencing datasets for which current methods fail. Our tools achieve highly accurate results with user-friendly installation, comprehensive documentation, efficient computation times and minimal memory usage.

We also applied our software tools for studying the crossover regulation role of FANCM in mammals. FANCM is a protein coding gene which encodes the protein that involves in DNA repair pathways and has been identified as a tumor suppressor. We used sgocaller and comapr on single-cell DNA sequenced mouse gametes for studying crossover regulation alteration in Fancm-deficient mice. We called crossovers from gametes collected from Fancm-deficient and wildtype mice for comparing the crossover frequencies estimated from two gamete populations. We observed that the number of meiotic crossovers was significantly increased in gametes collected from Fancm-deficient mice.

scATACPop: Simulating population scale single-cell ATAC seq data

Sagrika Chugh[1,2,3], Heejung Shim[2,3], Davis McCarthy[1,2,3]

[1]Bioinformatics and Cellular Genomics, St. Vincent's Institute of Medical Research, Melbourne, VIC, Australia [2]School of Mathematics and Statistics, The University of Melbourne, Melbourne, VIC, Australia [3]Melbourne Integrative Genomics, Faculty of Science, The University of Melbourne, Melbourne, VIC, Australia

Single-cell sequencing technologies have evolved and expanded rapidly in recent years, accompanied by the successful development of numerous computational tools. Single-cell Assay for Transposase-Accessible Chromatin sequencing (scATAC-seq) is a powerful method for genome-wide analysis of open (accessible) chromatin regions within individual cells derived from heterogeneous populations. Robust evaluation is critical for the development of scATAC-seq data analysis workflows, which are currently far from stable or mature. Notwithstanding important existing software tools, workflows remain in flux with many open questions regarding the best ways to optimize all elements of data analysis. Simulations are a crucial means for generating reproducible datasets with known characteristics that enable successful development, testing, and benchmarking of analysis methods. However, current scATAC-seq simulation frameworks do not fully account for the biological and technical characteristics of scATAC-seq data and are incapable of simulating population-scale scATAC-seq data with realistic genetic effects on chromatin accessibility and overall population structure. We present the scATACPop framework, a model and software tool for flexible and reproducible simulation of population-scale scATAC-seq data. We develop a new data generative model for scATAC-seq data that enables simulation of datasets that closely resemble real scATAC-seq datasets in library size, sparsity, and chromatin accessibility signals. scATACPop can also mimic complex batch, cell group, and conditional effects between individuals from different cohorts. The inclusion of genetic effects in our simulation framework will establish scATACpop as a key tool for the simulation of population scale scATAC-seq data generally, and, specifically, the development of methods for single-cell multi-omic quantitative trait locus mapping.

Uncovering subtype specific intra-tumoural heterogeneity in brain cancer

Saskia Freytag [1,2], Joel Duncan Moffet [1,2], Jordan Jones [3], Andrew Morokoff [3], Samuel Roberts-Thomson [4], Anna Pavenko [5], Alison Vanschoiack [5], James Whittle [1,2,6] and Sarah Best [1,2]

[1] *Personalised Oncology Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia;*
[2] *Department of Medical Biology, University of Melbourne, Melbourne, Australia;*
[3] *Department of Surgery, Royal Melbourne Hospital, Melbourne, Australia;*
[4] *Department of Anatomical Pathology, Royal Melbourne Hospital, Melbourne, Australia;*
[5] *NanoString Technologies, Technology Access Program, Seattle, USA;*
[6] *Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia*

Brain cancer is a deadly disease with a treatment regime that remains unchanged for decades. In brain cancer biology, intra-tumoural heterogeneity (ITH) is fast becoming an essential element to understand the complex nature of solid tumours. With the advent of single cell sequencing, tumours that appear homogenous by pathology are revealing unforeseen heterogeneity in the tumour sample. Indeed, in high-grade glioma (i.e., glioblastoma, GBM), increased ITH is associated with more aggressive disease. The next step in investigating ITH is to spatially discern these clusters of transcriptionally distinct cell types to identify specific niches in which they reside and cells they associate with. To investigate spatial ITH of brain cancer, we have obtained surgical brain tumour samples from low-grade (IDH1-mutant) and high-grade (IDH1-wt) glioma patients. Though routine histological analysis of these samples indicates IDH1-mutant low-grade glioma are homogenous, we have evaluated spatial regions of interest with increased cycling cells (Ki67 staining) and immune infiltrate (CD45 staining) by spatial whole transcriptome analysis. This analysis suggests that there are deeper levels of heterogeneity in these samples than initially appreciated, likely involving cell-to-cell communication with spatial significance. Indeed, the mesenchymal-like IDH1-wt glioblastoma displayed immune infiltrate that was enriched with macrophages, unlike IDH1-mutant counterparts. Together, this study reveals increased ITH in high-grade glioma, and a diverse immune landscape between glioma subtypes, likely impacting response to immunotherapy treatments.

Mapping cell-type specific transcriptomic signatures and defining differences between genetic subtypes in brain tissues of individuals with Prader-Willi syndrome.

Shokouh Shahrokhi Sabzevar[1], Candice Dyson[1], Mirana Ramialison[1], Michael See[1], Olivia Veatch[2], Anthony J. Hannan[3], David Amor[1], Merlin G. Butler[2], David Godler[1]

[1]Murdoch Children's Research Institute, Melbourne, Parkville, Australia
[2]University of Kansas Medical Centre, Kansas City, United States
[3]Florey Institute of Neuroscience and Mental Health, Parkville, Australia

We have established a cell-type specific differential expression map of protein-coding genes at 15q11-q13 in Prader Willi Syndrome (PWS) prefrontal cortex using the 10X Genomics Chromium system for single nucleus RNA sequencing (snRNA-seq). Brain samples were selected from the NIH-funded brain bank from: 4 individuals with PWS due to deletion (ages 31 to 52 years; 50% male), 4 individuals with PWS due to non-deletion (ages 31 to 44 years; 25% male) and 4 neurotypical controls (ages 32 to 42 years; 50% male). Significant differences were identified between these groups for the 34 protein-coding genes at 15q11-q13 for: (i) 26 comparisons of normalized number of transcripts expressed from these genes in each cell; (ii) 161 comparisons of proportion of cells expressing specific genes in different cell types. The imprinted genes (MKRN3, MAGEL2, NDN, NPAP1, SNRPN-SNURF and PWAR6) showed significant decrease in the proportion of cells expressing these genes for all cell types in PWS brain tissues when compared to controls (-Log(P) = 2.1 to 2.8). Significant differences were observed between deletion and non-deletion groups in non-neuronal cell types for UBE3A, HERC2, ATP10A and OPUD7A genes (-Log(P) = 1.5), and in the neuronal cell types for GABRB3 and GABRA5 (-Log(P) = 1.5). Of the non-imprinted genes, FAM189A1 and NSNMCE3 showed the greatest increase in proportion of cells and in normalized number of transcripts per cell expressing these genes in PWS as compared to controls for most cell types (-Log(P) = 2.1). In contrast, KLF13, OTUD7A and CHRNA7 expression showed significant PWS-specific changes, but only in neurons (-Log(P) = 1.1 to 1.9). Understanding how dysregulation of these genes affects different cell types of the brain in different subtypes, may lead to development of new: (i) disease models for PWS to test novel therapies; (ii) prognostic tests, enabling early intervention and targeted treatments.

Identifying gene fusions in single-cell RNA sequencing of cancers.

Shuyi Wu[1,2], Nadia Davidson[1,2]

[1]University of Melbourne, Melbourne, Australia
[2]Walter and Elizabeth Hall Institute, Melbourne, Australia

Gene fusions are genomic structural rearrangements that result in a fusion between two different genes. They can occur in cancer and have been found to act as driver mutations that can lead to oncogenic effects. Currently, fusion finding from short-read bulk RNA-sequencing data is well established and numerous fusion finding programs such as Arriba, STAR-Fusion and JAFFA have been written. With the advancement of single-cell sequencing technologies, cancer can be analysed at a finer resolution, such as subclonal classification. There is an opportunity to also identify fusions in single-cell transcriptome sequencing data. However, fusion finding tools for bulk data lack sensitivity when applied to high throughput single-cell data due to issues such as the low quantity of reads per cell and transcript end-bias of the read coverage. A new fusion finder, Fuscia (developed by Foltz et al.) is specifically made for single-cell (sc) RNA-seq data. It is unique in the way that it uses duplicates, i.e., reads with the same Unique Molecular Identifier (UMI) and cell barcode, which map to two different genes, to identify gene fusions.

In this project, we will present methods and preliminary results to compare different gene fusion finding programs for scRNA-seq data. We will use scRNA-seq data from several cancer cell lines where fusions have been well characterised, to benchmark the sensitivity and limitations of different fusion finders with respect to each other, with respect to bulk RNA-Seq and against fusion finding using new long read scRNA-seq protocols. We plan to assess sensitivity as a function of fusion expression levels, breakpoint locations relative to the 3' end of genes, and UMI duplication rates. Findings from this project will inform experimental design and future fusion finding methods development for single cell studies.

Single-nucleus Transcriptome Profiling of Kidneys Undergoing Compensatory Growth

Soon Wei Wong[1,2], Darling Rojas-Canales[1,2], Elise J Tucker[2], Jonathan M Gleadle[1,2]

[1]Department of Renal Medicine, Division of Medicine and Critical Care, Flinders Medical Centre
[2]Flinders Renal Laboratory, College of Medicine and Public Health, Flinders University

Compensatory renal hypertrophy (CRH) is a naturally occurring phenomenon seen when one kidney is removed, often for the purposes of kidney donation or removal of a renal cancer. The remaining kidney can grow by approximately 30% and improve its physiological function and this growth is dominated by increase in individual kidney cell size. However, the growth trigger, the early transcriptional program and role of particular cell types in the kidney during CRH remains unknown. C57BL6 mice (male, 10-weeks old) underwent nephrectomy of the left kidney (unx) or sham operation. Cell nuclei were isolated from a cross-sectional slice of the remaining kidney after 4hr (n=1 per group) and 24hr (n=2 per group) following surgery. Using 10x genomics single cell sequencing technology we comprehensively characterised transcriptional changes of the types of cells at single-nucleus level in the remaining kidney. Unsupervised clustering analysis of 24,657 nuclei from the snRNAseq data in the kidney revealed 34 cell types. Interestingly at 4h after kidney removal the majority of differential expression (DE) genes being upregulated, when compared to the 24h. Both timepoints however showed proximal tubule (PT) cells to have the largest number of DE genes in the nephrectomy (unx), when compared to Sham. Unx specific PT cells showed a significant increase of oxidative phosphorylation related genes. At 24h after nephrectomy, the medullary thick ascending limb (MTAL), was the cell type with the next most substantial transcriptional changes, and showed increases in genes involved in cholesterol metabolism and mTORC1 signalling. Firstly, we revealed key cell type-specific transcription factors and major gene-regulatory circuits for kidney cells undergoing CRH. PT cells showed the greatest change when compared to other cell types, potentially to meet the energy needs of sodium reabsorption after losing a kidney. Secondly, we demonstrate that the generation of comprehensive high-resolution, single-nucleus transcriptomic profiles of CRH mice provides resources to identify kidney growth related genes and pathways that could be co-opted therapeutically.

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Inferring cell-specific causal regulatory networks from drift and diffusion

Stephen Zhang[1], Michael Stumpf[1]

[1]*University of Melbourne*

Cellular dynamics are fundamentally governed by networks of interacting genes, and inferring these interactions from data is a central problem in systems biology. Gene expression profiling at single-cell resolution is now routine, allowing for heterogeneity of cell state to be studied at scale. In contrast, the vast majority of network inference methods work at the population level to construct a static gene regulatory network, and thus do not allow for inference of differential regulation across sub-populations. Building upon recent inference methods that model single cell dynamics using drift-diffusion processes, we propose a method for inferring cell-specific causal networks and show some preliminary results.

Synthetic single-cell tools to decipher clonal fate programs

Tom Weber [1], Christine Biben [1], Denise Miles [1], Mathew Chu [1], Sara Tomei [1], Samir Taoudi [1], Shalin Naik [1]

[1] *WEHI, Melbourne, Australia*

Fate programs are crucial for any multicellular organism to reproduce, develop, renew and protect itself against pathogens. Such programs are an amalgamation of cellular replication and cell fate programming. Traditionally, fate programs have been studied at a population level, and more recently on the single cell level. Here I put forward the proposal that fate programs should be studied at the clonal level and discuss examples where this approach in combination with synthetic biology tools has provided new insights. I will then give an update on the LoxCode technology, and how we are employing this novel in situ barcoding tool to query clonal fate heterogeneity during mouse embryogenesis. I will conclude with an outlook towards the next generation of cellular transcriptional recorders that have the potential to link the past, present and future at the single cell and clonal level.

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Generation of spatial transcriptomics data from skin cancer and melanoma fixed tissues to add molecular signatures to pathological annotation

Tuan Vo [1], Kahli Jones [1], Pui Yeng Lam [1], Joanna Crawford [1], Yung-Ching Kao [2], Peter Soyer [2], Kiarash Khosrotehrani [2], Mitchell Stark [2], Quan Nguyen [1]

[1] *The Institute for Molecular Bioscience, the University of Queensland*
[2] *Diamantina Institute, the University of Queensland*

Almost all suspected cancer patients have tissue biopsies examined by pathologists using light microscopy. Spatial transcriptomics (ST) is an emerging technology that can produce both a histopathology-grade ST, thereby, adds spatial information of gene expression to traditional histopathological images. Such technology has the potential to revolutionise our current understandings of tissue biology, especially cancer tissue. However, technical variations in accessing and permeabilising cells across different tissue types and tissue areas cause difficulties in performing high-quality ST for cancer tissues. Notably, a vast amount of unexplored archival cancer tissue samples are formalin-fixed paraffin-embedded. Making the best use of the precious cancer tissue resource would make breakthroughs needed in understanding the cancer cellular environment and how the diverse cells can be used as markers to inform diagnosis, treatment regimes and to predict outcomes. Notably, measurement of gene expression from these tissues has been highly challenging due to several critical technical issues, such as RNA retrieval from the cross-linked and partially degraded RNA source with the tissue.
We optimised ST protocols to generate unprecedented spatial gene expression data for FFPE skin cancer. These are among the most challenging tissue types to perform ST due to their fibrous structure and a high risk of RNAse contamination. We evaluated tissues collected from ten years to two years ago, spanning a range of different tissue qualities and complexity. Samples from patients with different survival outcomes, cancer types, and cancer stages were compared. Further, we overlaid gene expression profiles with pathological information, revealing a new layer of molecular information that can reveal gene markers unique for cancer cells, locations and stages. Together, this work provides important technical perspectives to enable the applications of ST on cancer tissues. In addition, we present a pipeline for integrating molecular transcriptomics with histopathological analysis in cancer research and clinical applications.

scMoE: A regularized mixture of experts model to identify heterogeneous relationship between cell type proportion and host health in COVID-19 patients.

Xiangnan Xu [1]

[1] *School of Mathematics and Statistics. The University of Sydney. Camperdown, NSW, Sydney, Australia.*

The advent of single-cell technology has enabled an unprecedented understanding of the behavior of individual cells. Numerous studies have found that cell proportion is closely related to human health outcomes. However, such relationships are heterogeneous at an individual level and may vary among populations. Bulk RNA sequencing provides insight into the gene expression profile of an individual and could be useful to divide populations into subcohorts with similar cell proportions and health outcome relationships. In this study, we present a novel single-cell Mixture of Experts model for establishing associations between cell proportion and health state that accounts for the variability of individuals' gene expression profiles. By jointly optimizing the latent class of individuals with different bulk expression profiles and relationships between cell proportion and health outcome, scMoE can identify heterogeneous relationships between cell proportion and host health. We applied scMoE to a COVID-19 dataset of 153 patients in two states (mild and severe) and identified two latent classes described by the bulk gene expression and/or individual characteristics. We found that the relationship between CD8+T cell and COVID-19 disease progression is reversed between these two latent classes, illustrating the potential of scMoE to shed light on the individual heterogeneous response of COVID-19.

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A novel computational approach for integrative analysis of spatial multi-omics data highlights immune responses to SARS-CoV-2 infection in lung tissue

Xiao Tan [1], Laura F. Grice [1,2], Minh Tran [1], Onkar Mulay [1], Guiyan Ni [1], Anna Flavia Robeiro dos Santos Miggiolaro [3], Jarbas da Silva Motta Junior [3], Karen Fernandes de Moura [3], Clever Machado de Souza [4], Cristina Pellegrino Baena [3], Lucia de Noronha [6], Hung N. Luu 78, Caroline Cooper [3], James Monkman [3], Kirsty Short [9], Gabrielle Belz [3], Fernando Souza-Fonseca-Guimaraes [3], Arutha Kulasinghe [3]*, Quan Nguyen [1]*

[1] *Institute for Molecular Bioscience, The University of Queensland*
[2] *School of Biomedical Sciences, The University of Queensland*
[3] *University of Queensland Diamantina Institute, Faculty of Medicine, The University of Queensland, Woolloongabba, QLD 4102, Australia.*
[4] *Hospital Marcelino Champagnat, Programa de pós-graduação em Ciências da Saúde (PPGCS) da PUCPR*
[5] *Laboratório de Patologia Experimental, PPGCS da PUCPR*
[6] *Instituto de Pesquisa Pelé Pequeno Príncipe - Faculdades Pequeno Príncipe*
[7] *UPMC Hillman Cancer Center, University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA, USA*
[8] *Department of Epidemiology, School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA*
[9] *School of Chemistry and Molecular Biosciences, The University of Queensland*

The current boom in spatial sequencing and imaging technologies has increased the potential data which any research group can generate, yet cross-comparison between data modalities remains a computational and conceptual challenge.

We sought to develop a novel computational strategy using tissue morphology to map spatial-omics data from different modalities into a common tissue coordinate framework, which allows complementary protein and/or RNA data to be analysed together, facilitating cross-validation between modalities. We then applied our approach to comprehensively characterise the transcriptome and targeted proteome of COVID-19 infected viral infected and uninfected tissues.

For each of five technologies (Visium, CODEX, Nanostring GeoMx, Polaris and RNAscope), we generated complimentary spatial-omics data for tissue microarray blocks from lung biopsies with or without COVID-19 infection. After mapping spatial transcriptomics (Visium) and proteomics (CODEX) data, we uncovered high correlation between protein and RNA expression in smooth muscle and airway epithelial cells. Moreover, both transcriptomics data (Nanostring) and proteomics data (CODEX and Polaris) showed increased immune activity in COVID-19 infected samples compared to non-infected samples, with an increase in NK cells and T cells identified in infected samples across modalities. When comparing tissues with variable SARS-CoV-2 viral loads, as determined by RNAScope, we observed an increased signature of macrophage infiltration in tissues with a higher viral load, as well as an enrichment of cytokine and interferon responses and chemokine signalling in these areas of increased viral load.

In summary, this study presents a novel integrated approach for the combination of spatial multi-omics data which can be applied to any combination of data types for which imaging data is available. Our case study in SARS-CoV-2 infected tissues highlights this approach as a novel way to study immunological responses in tissue context, making use of both spatial transcriptomic and proteomic data.

Improving UMAP by preserving feature information enhances single cell data analyses

Yang Yang

Diamantina Institute - University of Queensland, Brisbane, Australia

Dimensionality reduction methods, especially non-linear methods like UMAP, have been ubiquitously utilized in single cell data analysis for visualization, pseudo-time inference and data integration. The non-linear methods can effectively maintain local structure of high dimensional data to reveal the clustering structure. One essential limitation of nonlinear dimensionality methods is losing the feature information of high-dimensional data. In this paper, we design feature and transition enabling manifold approximation and projection (fateMAP) to preserve feature in low dimensional embedding space. Compared to conventional differential gene expression analysis among clusters, fateMAP maintaining feature information can demonstrate the most locally variable genes. Mathematically, we illustrate that the feature information captures local variability which corresponds to the limit of differentially expressed gene analysis. By applying fateMAP to single cell RNA sequencing data of CD8 T cells, we utilize feature information to define the core states of naïve T cells and effective T cells. For each core state, we compute the optimal cell-fate transition directions and derive most probable path among states, which facilitates to define the cell fate from naïve CD8 T cells to effective T cells. Along the path between core states of naïve and effective T cell, we cluster the gene expression trends and gene variability change to detect the driving genes and compute gene regulation patterns. The novel gene regulation patterns can help to understand the gene differentiation mechanism of CD8 T cell.

Oz Single Cell

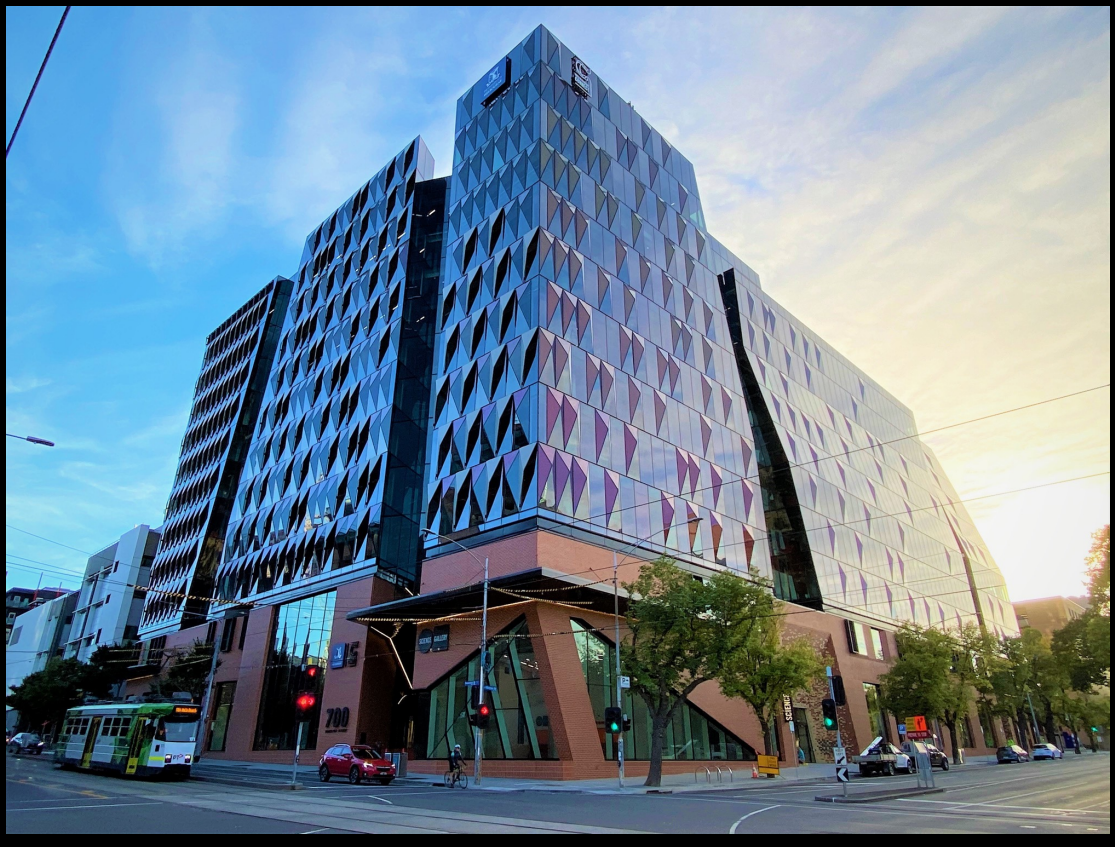
We'll see you next year!

That's all from us this year, but we look forward to meeting again in Melbourne next year for **OzSingleCell23**!

When: 25-27th October, 2023

Where: Melbourne Connect, University of Melbourne

Theme: Clonal Biology



In the meantime, keep updated on twitter @ozsinglecells and our website singlecells.org.au!