The 6th OzMRS Scientific Symposium

1st November 2023
Ian Potter Auditorium, Melbourne Brain Centre
University of Melbourne

ABSTRACT
BOOKLET

@ozmrs1

We acknowledge the Wurundjeri people of the Kulin Nation, as the Traditional Owners of the land on which we meet for this conference. We pay our respects to the Elders past and present and extend our respect to any First Nations people joining us today.

About the OzMRS

The OzMRS is the Australasian Chapter of the International Metastasis Research Society (MRS). The OzMRS was formally established in 2013 by co-founding members, Prof Rik Thompson (Queensland University of Technology, QLD) and Prof Robin Anderson (Olivia Newton-John Cancer Research Institute, VIC), and the local organising committee of the MRS congress convened in Brisbane, 2012. The overarching goal of the OzMRS is to build an engaging society that embraces all with an interest in metastasis.

Our Mission

- Our mission is to act as a central organisation that connects all stakeholders with an interest in metastasis, including laboratory scientists, clinical researchers, industry, consumers and advocacy groups, and to promote the world-class metastasis research from the Australasian region. To achieve this, we aim to:
- Actively promote scientific events and disseminate the latest research and treatment advances through our dedicated OzMRS website and social media.
- Convene biennial metastasis-focused scientific symposia to facilitate the exchange of information, foster collaboration between all stakeholders and enhance the engagement of Australasian metastasis researchers, both nationally and internationally.
- Mentor and champion students, early and mid-career researchers by supporting their integration and participation in metastasis-focussed scientific events.
- Engage with, and educate the broader community about the need for metastasis research through active partnership with consumers and advocacy groups.

Our Vision

We are committed to promoting all aspects of metastasis research that will improve the quality of life of individuals with advanced disease. We will support and provide opportunities for researchers whose work seeks to advance world-class research towards improved monitoring, prevention and treatment of metastasis.

We are proud that the 2023 OzMRS Symposium has 'Consumers Included' status provided by Cancer Voices ensuring that the perspectives of patients, survivors, carers and other members of the public affected by cancer are part of the planning and program of the conference.

www.ozmrs.com

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Why include consumers?

The added value of including informed consumers in conferences, forums and other events, for the organiser, the attendees, representative consumers and their networks is well accepted.

- Involving consumers ensures that the research and discussions are aligned with the needs and perspectives of those directly affected by cancer. This can lead to more patient-centred care and research, which ultimately benefits patients and survivors.
- Consumers can provide firsthand experiences and insights into the challenges and issues they face during their cancer journey. These real-world perspectives can guide researchers and clinicians in identifying gaps in care and improving the quality of life.
- The involvement of clinicians allows for a more comprehensive view of cancer care and treatment. Clinicians can provide insights into the practical challenges of implementing research findings in clinical settings and share their expertise on the latest treatment options.
- Engaging with consumers fosters a sense of community and support. It also helps participants feel more connected to the research and healthcare community.
- Including consumers can help address ethical concerns in research, such as informed consent, privacy, and data sharing. These individuals can offer input on how research is conducted and ensure that it aligns with ethical standards.
- Involvement in such events empowers consumers to advocate for their needs and rights.
- Scientific symposiums bring together professionals from various fields. Including consumers encourages collaboration between researchers, clinicians, and patients/survivors, fostering a multidisciplinary approach to cancer care and research.
- The active participation of consumers can lead to more relevant research questions, more effective study designs, and better communication of research findings to the public.

Overall, involving consumers in scientific symposiums enhances the quality and relevance of the discussions, promotes patient-centred care, and ultimately contributes to the overall goal of improving cancer care and outcomes.

How can we improve the integration of consumers in metastasis cancer research?

This will be discussed as part of the conference – see page 28 for more information.

OzMRS Committee

Prof Robin Anderson	*Founding Member, Conference Organiser*
Prof Rik Thompson	*Founding Member, Conference Organiser*
A/Prof Delphine Merino	*President, Conference Organiser*
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6th OzMRS symposium

1st November 2023

Ian Potter Auditorium, Melbourne Brain Centre

University of Melbourne

Welcome

8:45-9:00	Arrive (collect badges and late registrations)
9:00-9:10	Opening and Welcome, Delphine Merino, President OzMRS

Session 1: AlphaXRT Sponsored Session

Session Chairs: Kathryn Leaney and Adrian Wiegmans

9:10-9:40	Keynote speaker: Hyun Woo Park (Henry), Yonsei University, Korea *'Reprogramming Anchorage Dependency by Adherent-to-Suspension Transition Promotes Metastatic Dissemination'*
9:40-9:55	Peter Croucher, Garvan Institute, Sydney *'Dissecting Myeloma Cell Dormancy One Cell at a Time'*
9:55-10:10	Suresh Mathivanan, La Trobe University, Melbourne *'Exercise plasma-derived extracellular vesicles as metastasis modulators'*
10:10-10:25	Sarah Boyle, University of South Australia, South Australia *'Compressive force-induced mechanotransduction promotes breast cancer progression'*
10:25-11:00	Morning Tea

Session 2

Session Chairs: George Kiossoglou and Charlotte Roelofs

11:00-11:30	Keynote speaker: Liz Caldon, Garvan Institute, Sydney *'The slow road to metastasis in ER+ breast cancer'*

11:30-11:45 Bhupinder Pal, Olivia Newton John Cancer Research Institute, Melbourne

'The circulating immune profile stratifies metastatic burden in breast cancer patients'

11:45-12:00 Xiaowen Liang (Tina), University of Queensland

'Identification and characterisation of tumour self-seeded cells'

12:00-12:45 OzMRS AGM

12:45-2:00 Lunch and Poster Session

Session 3

Session Chairs: Elizabeth Williams

2:00-2:30 ECR 3 min flash talks and interpretation from consumers

Chamikara Liyanage, Olivia Newton-John Cancer Research Institute, Melbourne

'Improving Immunotherapy Treatment Response in Advanced Breast Cancers'

Sreeja Gadipally, Olivia Newton-John Cancer Research Institute, Melbourne

'Identifying aggressive clones in metastatic breast cancer models'

Terrance Lam, Monash University, Melbourne

'*HOXC12: The Master Regulator of β2-Adrenoceptor-Mediated Invasion in Triple Negative Breast Cancer*'

Yesha Ramani, University of South Australia, Adelaide

'The RNA binding protein Quaking regulates prostate cancer cell plasticity by influencing widespread changes in alternative splicing'

Michael Trpceski, Garvan Institute of Medical Research, Sydney

'Pinpointing and targeting novel drivers of pancreatic cancer progression and metastasis using TRAP-seq'

Shruti Tushar Deshpande, University of South Australia, Adelaide

‘*A novel RNA binding protein (ZCCHC24) as a regulator of cancer cell plasticity’*

2:30-2:45 Perspective of metastasis research (Robin Anderson and Rik Thompson)

2:45-3:30 Panel Discussion: Consumers, Clinicians, Researchers (Big Qs -Q&A)

Professor Daniel Brungs (Medical Oncologist, University of Wollongong)

Ms Kathryn Leaney (Executive Committee, Cancer Voices NSW)

Professor Renae Taylor (Cancer Program, Monash University)

Associate Professor Niall Corcoran (Surgeon and Research Fellow, University of Melbourne)

Mr Graeme Sissing (Cancer Research Consumer Advocate)

3:30-3:45 Afternoon Tea

Award Session

Session Chairs: Graeme Sissing and Tom Cox

3:45-4:45 Selected student/ECR talks for prizes (8 min talks + 2 min Qs)

Aeson Chang, Monash Institute of Pharmaceutical Sciences, Melbourne

‘*Triple negative breast cancer hijacks the sympathetic nervous system to resist chemotherapy’*

Brooke Pereira, Garvan Institute of Medical Research, Sydney

‘Temporal proteomics and advanced microscopy reveal nidogen-2 as a new stromal target in pancreatic cancer’

Charlotte Roelofs, Olivia Newton-John Cancer Research Institute, Melbourne

‘MYC as a master regulator of dormancy in triple negative breast cancer’

Moganalaxmi Reckdharajkumar, Adelaide Medical School, University of Adelaide

‘Investigating the Role of Rho-ROCK Signalling in Breast Cancer Metastasis’

Annabel Manoleras, Monash Institute of Pharmaceutical Sciences, Melbourne

'Nerves talk to metastases: Characterising spatial relationships between nerves and metastatic cancer cells'

Ashna A. Kumar, University of Wollongong

'A "peak" into the intratumoural distribution of a novel, small-molecule anti-metastatic agent via mass spectrometry imaging in human pancreatic ductal adenocarcinoma'

4:45-5:00 Talk by Platinum Sponsor – Alpha XRT

5:00-5:30 Award Presentation

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AlphaXRT Sponsored Session

Keynote Speaker: Hyun Woo Park (Henry), Yonsei University, Korea

Reprogramming Anchorage Dependency by Adherent-to-Suspension Transition Promotes Metastatic Dissemination

Henry is currently the Director of AST Metastasis Research Center funded by the National Grand Challenges Initiative. He pioneered a novel paradigm referred to as Adherent-to-Suspension Transition (AST) which reveals the role of hematopoietic transcription factors in CTC generation and metastasis. His current research is focused on the development of next generation anti-metastatic strategies that target the AST factors in CTCs.

Professor Peter Croucher, Garvan Institute, Sydney

Dissecting Myeloma Cell Dormancy One Cell at a Time

Peter trained at the University of Wales College of Medicine and Cambridge and Oxford Universities in the UK. In 2003 he joined Sheffield University where he was Head of Department of Human Metabolism. In 2011 Peter joined the Garvan Institute of Medical Research in Sydney where he is now Director of the Cancer Plasticity and Dormancy program. Peter's interests are in cancers that grow in the skeleton, understanding cancer induced bone loss and investigating cancer cell dormancy and disease relapse.

Suresh Mathivanan, La Trobe University, Melbourne

Exercise plasma-derived extracellular vesicles as metastasis modulators

Suresh Mathivanan undertook a PhD in proteomics and bioinformatics at the Institute of Bioinformatics, India and Johns Hopkins University, USA. After PhD, Suresh joined Ludwig Institute for Cancer Research, Melbourne, Australia as a postdoctoral researcher. In 2011, he received a NH&MRC Peter Doherty fellowship to study exosomes secreted by cancer cells. In 2015, he received an ARC DECRA to study the role of exosomes in intercellular communication. Suresh moved to the Department of Biochemistry, La Trobe Institute for Molecular Science (LIMS) at La Trobe University after receiving a LIMS fellowship to set up his own research group in 2011. At LIMS, Mathivanan's laboratory is focused on exosomes, their role in cancer and intercellular communication. Currently, he is funded by an ARC Future Fellowship (FT2: 2018-2022) to investigate how exosomes are made up by cells. He has authored over 91 papers that are cited more than 17100 times (Google Scholar; Nov 2019).

Dr Sarah Boyle, University of South Australia, South Australia

Compressive force-induced mechanotransduction promotes breast cancer progression

Dr Sarah Boyle is an Australian Research Council DECRA Research Fellow at the Centre for Cancer Biology in Adelaide, South Australia. Sarah completed her PhD at the University of Adelaide, investigating the roles of chemokine receptors in mammary gland biology and breast cancer, before joining the Tumour Microenvironment Laboratory at the CCB. Sarah's research focusses on the mechanisms and interplay between mechanical force, mechanotransduction, and various aspects of the tumour microenvironment in progression and metastasis of breast cancer, with publications in multi-disciplinary journals including Nature Cell Biology, Journal of Cell Science, Advanced Science, and Oncogene. She has previously held fellowships from the Royal Adelaide Hospital Research Committee and Australian Breast Cancer Research through The Hospital Research Foundation, and is an emerging leader in her field.

Session 2

Keynote speaker: Liz Caldon, Garvan Institute, Sydney

The slow road to metastasis in ER+ breast cancer

A/Prof Liz Caldon is a lab head at the Garvan Institute of Medical Research in Sydney, Australia. She runs a basic and translational research laboratory on estrogen receptor positive (ER+) breast cancer, basal-like breast cancers and ovarian cancers. Liz's group has built models of hormone therapy and CDK4-6 inhibitor resistance to understand the evolution of ER+ breast cancers as they become refractory to standard of care therapies. Specific interests of her group are the understanding of tumour cell evolution through single cell modelling, cell cycle based therapies, therapy repurposing, and the development of targeted approaches in therapy resistant disease.

Bhupinder Pal, Olivia Newton John Cancer Research Institute, Melbourne

The circulating immune profile stratifies metastatic burden in breast cancer patients

Bhupinder Pal is a Victorian Cancer Agency Research Fellow and laboratory head at the Olivia Newton-John Cancer Research Institute (ONJCRI). Bhupinder obtained PhD degree from the University of Melbourne (2009) and postdoctoral training in the field of breast cancer biology at the Walter and Eliza Hall Institute (WEHI). He has received the NHMRC Peter Doherty Fellowship, NHMRC New Investigator grant and Victorian Cancer Agency Early and Mid-career fellowships. Dr Pal has extensively used single-cell methods to refine mammary epithelial lineage relationships, identify rare and intermediate epithelial sub-types and map molecular events that shape the breast tumour microenvironment. His current research focuses on understanding the role of tumour heterogeneity and stromal-immune cell crosstalk during cancer progression and identifying novel cancer immunotherapy targets.

Xiaowen Liang (Tina), University of Queensland

Identification and characterisation of tumour self-seeded cells

My research focuses on liver biology and pharmacology and I am a research team leader of the joint liver cancer research program of Frazer Institute and Gallipoli Medical Research Foundation from 2019. My long-term goal is to direct a research group exploring strategies to improve liver cancer outcomes in patients, working closely with clinicians to enable bench-to-bedside translation.

Research Impacts: I developed an intravital imaging platform for visualising and characterising the spatial distribution of activated hepatic stellate cells and metabolic status in liver cancer. This imaging platform and physiologically based kinetics modelling was applied for the first time to investigate the sub-organ disposition and clearance of therapeutic agents in the liver. By expanding the initial work, I further led studies on assessment and monitoring of cellular oxidative stress, microcirculation, and drug response in the specific liver microenvironment for early prediction of treatment efficacy in liver diseases.

Poster Session

Rebecca Brown: Investigating the role of γδ T cells in metastatic breast cancer

Rebecca Brown1,2, Stefano Mangiola3, Kok Fei Chan1,2, Shalini Guleria1,2, Caroline Bell1,2, Simone Ostrouska1,2, Robin L Anderson1,2, Lisa Mielke1,2, Andreas Behren1,2, Bhupinder Pal1,2
1 Olivia Newton-John Cancer Research Institute
2 School of Cancer Medicine, La Trobe University
3 Walter Eliza Hall Institute

Abstract
γδ T cells have been shown to play an important role in the tumour microenvironment. In general, these cells are cytotoxic and capable of killing cancer cells, however, their role during metastatic disease progression is not fully understood. In this study, we investigated the two major γδ T cell subsets (Vδ1 and Vδ2) in metastatic breast cancers. We aim to determine how these subset act and react in metastatic progression of breast cancer.

We interrogated over 35 breast cancer specimens using multiplex immunohistochemistry (mIHC) to score tumour immune infiltrates including γδ T cells. These analyses determined that γδ T cell frequency is tightly correlated with the breast cancer stage. Compared to primary tumours, the γδ T cell subsets are relatively depleted in metastatic tissues, suggesting that these cells are differentially regulated at metastatic sites. Additionally, we interrogated the function and the phenotypic properties of γδ T cells in in vitro tumour-killing assays utilising breast cancer cell lines with differential metastatic potential. We found that γδ T cells kill breast cancer cells with varying efficiency that is inversely correlated to the metastatic potential of the cell lines. Collectively, we see a decrease in the prevalence of γδ T cells in the metastatic environment and an increase in resistance to γδ T cell-mediated killing in the metastatic cells, contributing to uncontrolled growth in metastatic lesions.

Overall, this study aims to exploit γδ T cell properties for designing novel treatments against metastatic breast cancer.

Ekaterina (Kate) Ivanova: Using an in vitro model of Circulating Tumour Cells to decipher adaptational changes in DNA Repair in Chemoresistant Breast Cancer Cells.

Ivanova E1, Parker AL2, Cox T2, Fernandez Canizalez A1, Richard DJ1, O'Brien K1,3, Wiegmans AP1.

1 Cancer and Ageing Research Program, Centre for Genomics and Personalised Health, Queensland University of Technology, Woolloongabba, QLD, 4102, Australia.
2 Matrix and Metastasis Lab, Kinghorn Cancer Centre, Garvin Institute of Medical Research, Darlinghurst, NSW, 2010, Australia.
3 Princess Alexandra Hospital, Oncology, Woolloongabba, QLD, 4102, Australia.

Abstract

Background: Dysregulated DNA damage repair (DDR) generates a variety of genetic variants increasing the likelihood of emergence of resistant phenotypes [1]. Therefore, genotoxic pressure from neoadjuvant chemotherapy could potentially prime the tumour to become not only resistant to subsequent treatment but also more invasive leading to metastatic spread [2]. There is a paucity of understanding what role DNA damage response plays in maintenance of circulating tumour cells (CTCs) and how it enables metastasis formation.
Aims: To investigate how breast cancer CTCs respond to DNA damage and whether development of chemoresitance supports or impedes cells' transition to suspension/non-adherent conditions.
Methods: We developed a model of CTCs by adapting triple-negative breast cancers cell lines to suspension culture. We evaluated cells' response to inhibition of key mediators of double strand repair, assessed functional variations in DNA repair dynamics.
Results: Consistent with our previous work on chemoresistance in triple-negative breast cancer, our results indicate that cells' transition from adherent to suspension state is accompanied by a shift in DDR pathway of choice.
Conclusions: Cancer progression from primary tumour to CTCs to metastasis is associated with adaptational changes in DNA repair response.

Alastair Saunders: TGF-β1 induces metastatic colonisation to skeletal muscle in mouse models of metastatic breast cancer

Alastair A.E. Saunders1, Rachel E. Thomson1, Kellie E. Mouchemore2,3, Chris Karagiannis1, Lauren S. James1, Thomas B. Chadwick1, Adam Hagg1, Hongwei Qian1, Allan D. Burrows2,3, Robin L. Anderson2,3,4, Paul Gregorevic1,5

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2 Metastasis Research Laboratory, Olivia Newton-John Cancer Research Institute, Heidelberg, VIC, 3084, Australia.
3 School of Cancer Medicine, La Trobe University, Bundoora, VIC, 3086, Australia.
4 Peter MacCallum Cancer Centre Melbourne, Victoria, 3000, Australia.
5 Department of Neurology, The University of Washington School of Medicine, Seattle, Washington, 98195, USA

Abstract
Cancer metastasis occurs by tumour cell dissemination throughout the bloodstream and lymphatics to distant organs. Selectivity exists between different tissues and cancers that dictates where metastases will develop. Common sites of metastasis including the lymph nodes, lung, liver, bone and brain. Intriguingly, skeletal muscles are rarely the site of secondary tumour growth despite making up 30-40% of a human's body mass and receiving a rich blood supply. The mechanism underlying why skeletal muscle has an apparent resistance to metastasis remain unclear.
This research aims to understand why muscles are infrequently affected by metastatic cancers. Based on previous studies showing TGF-β as a regulator of cancer cell dissemination and colonisation, we hypothesised that manipulation TGF-β in muscle would render it more susceptible to metastasis. To test this hypothesis we increased expression of TGF-β1 through gene delivery locally in mouse muscles with concurrent orthotopic inoculation of mCherry-labelled 4T1.2 breast cancer cells. Mice harbouring lung metastases were assessed for evidence of cancer cells in muscles by histology, qPCR and flow cytometry.
Histological analysis and qPCR identified mCherry-positive tumour cells in TGF-β expressing but not control muscles. Furthermore, we found that colonisation was associated with an increase in immune cells, and that skeletal muscle colonisation occurred to a lesser extent in immunocompromised mice.
These results demonstrate that altering the muscle microenvironment through TGF-β1 promotes colonisation and growth of cancer cells. Successfully defining the unique factors within muscles that deter metastatic propagation may identify potential anti-metastatic agents that could prevent metastases in vulnerable organs.

Md Amir Hossain: MHC-II+ tumour-associated macrophages are present in responders of chemotherapy-beta-blocker combination therapy

Md Amir Hossain1, Sapna Devi2, Scott N. Mueller2, Aeson Chang1, Erica K. Sloan1

1. Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia
2. Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia

Abstract

Preclinical and clinical studies are exploring the utility of pharmacological beta-blockade as a novel strategy to enhance existing cancer treatments. Beta-blockers block signalling from the sympathetic nervous system and have been shown to improve the efficacy of anthracycline chemotherapy in controlling metastasis in mouse cancer models and breast cancer patients (Chang, et al., 2023, Sci Trans Med). However, not all patients show a favourable response. To better understand factors that contribute to treatment response, we profiled the immune landscape of tumours in responder and non-responder mice. While the overall recruitment of F4/80+CD64+ macrophages, and CD4+ and CD8+ T cells to the tumour microenvironment were similar between responder and non-responder mice, mice that showed resistance to chemotherapy-beta-blocker combination treatment had lower levels of MHC-II+ tumour associated macrophages in the tumour, compared to responders. MHC II+ M1-like macrophages have been shown to have a tumour suppressive effect, suggesting the levels of MHC-II+ M1 tumour associated macrophages infiltration may influence the efficacy of chemotherapy and beta-blockade therapy. Ongoing experiments are examining a causal role for this cell types in the treatment enhancing effects of beta-blockers on chemotherapy.

Keywords: sympathetic nervous system, chemoresistance, tumour-associated macrophages, Cancer metastasis

James Comben: Understanding the Emergence of Neuroendocrine Prostate Cancer Using Single-Nuclei RNA-Seq Data from Rapid Autopsy Samples

James Comben [1,2], Lachlan Cain [1], Sirui Weng [1,3], Sara Alaei [1], Timothy Semple [1], Luciano Martelotto [4], Rick Pearson [1], Luc Furic [1,3], Shahneen Sandhu [3,5], Anna Trigos [1,3]

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[3] Sir Peter MacCallum Department of Oncology, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne
[4] Adelaide Centre for Epigenetics, South Australia ImmunoGENomics Cancer Institute (SAIGENCI).
[5] Medical Oncology, Peter MacCallum Cancer Centre

Abstract
Single-cell technologies, such as single-nuclei RNA-seq, have opened new possibilities for understanding stages of differentiation during cancer development and treatment resistance in patient samples. A key challenge in the treatment of metastatic castration resistant prostate cancer (mCRPC) is the process of transdifferentiation from adenocarcinoma to a neuroendocrine prostate cancer (NEPC) phenotype. NEPC leaves patients with few treatment options and a median progression free survival of just 3.9 months. Understanding how adenocarcinoma cells transdifferentiate into NEPC is yet to be characterised in patients, as studies have traditionally been done in vivo and in vitro.

This project aimed to identify markers of early neuroendocrine differentiation using single-nuclei RNA-seq data from mCRPC patient tumour samples obtained via the Cancer Tissue After Death (CASCADE) rapid autopsy program at the Peter MacCallum Cancer Centre. Findings were independently validated in neuroendocrine prostate cancer datasets of bulk RNA-seq data and single cell sequencing data from patients and patient-derived xenografts.
We identified a trajectory for NEPC transformation that was recapitulated across multiple tumour samples from multiple metastatic sites per patient, and across patients. Gene signatures were identified for each stage of the trajectory including several gene candidates that may be involved in initiating the lineage switching process. Analysis in external datasets revealed the trajectory linked gene HEPACAM2 to be an early marker of NEPC differentiation.

Our findings provide several gene candidates for future NEPC transformation research that could potentially be used to identify patients at risk of developing NEPC, or potentially novel targets for treatments.

Chi Yau Liu: Long non-coding (lncRNAs) regulation in epithelial-mesenchymal transition

Chi Yau Liu1, Katherine A Pillman1 , Cameron P Bracken1
1.Centre for Cancer Biology, An Alliance of SA Pathology and University of South Australia, Adelaide, Australia

Abstract
Long non-coding RNAs (lncRNAs) are a class of RNA molecules defined by their length exceeding 200 nucleotides and by the lack of an open reading frame. As with other protein-coding mRNAs, they are transcribed by RNA-Pol II, possess a 5' m7 Cap and 3' poly-A tail, and undergo alternative splicing. Although previous studies have documented the existence of over 100,000 lncRNAs, only a fraction of them have been functionally characterized. Epithelial-mesenchymal transition (EMT) represents a reversible transformation of cells between epithelial and mesenchymal states which plays crucial roles in development, wound healing and metastasis. Extensive gene expression changes are associated with EMT, including lncRNAs, though few reports investigate the significance of such regulatory events. In this project, we have re-mapped bulk-sequencing datasets obtained from our own EMT model (comparing HMLE and mesHMLE cells) to investigate the regulation of lncRNAs across the EMT spectrum. We identified five highly expressed lncRNAs in epithelial cancer cells, which exhibited minimal expression in mesenchymal cells. Our analysis focuses on understanding the contributions of these lncRNAs to various aspects of EMT, including alterations in cell morphology, cell motility, and invasiveness.

Natalia Vukelic: GPX2 Loss Sensitises Colorectal Cancers To Radiotherapy And Chemotherapy

Natalia Vukelic1,2, Jennifer K Mooi1,3, Dmitri Mouradov4,5, Stan Kaczmarczyk1,4, Rebecca Nightingale1,2, Delphine Merino1,2, George Iatropoulos1,2, Niall Tebbutt1,2,6, Diego Arango7, Oliver Sieber4,5,6,8, Sefi Rosenbluh8, Ian Y Luk1,2,3, David Williams1,9 and John M. Mariadason1,2,3

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7. Molecular Biology and Biochemistry Research Center, University Hospital Research Institute, Barcelona, Spain.
8. Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia.
9. Department of Anatomical Pathology, Austin Health, Melbourne, Victoria, Australia.

Abstract

Background & Aim: Whilst chemotherapy and radiotherapy are major treatment modalities for colorectal cancers, not all patients benefit. Strategies which can enhance the efficacy of these mainstay treatments, and predictive biomarkers facilitating the stratification of patients likely to benefit – sparing them from surgery, possible colostomy, and unnecessary adjuvant chemotherapy - would represent a major clinical advance. Herein, we identified frequent epigenetic inactivation of the ROS-detoxifying enzyme Glutathione Peroxidase 2 (GPX2) in poorly differentiated CRCs; with decreased expression also observed amongst metastatic sites compared to primary tumours. Given GPX2 is the key detoxifier of reactive oxygen species within the colonic epithelium, this study sought to investigate whether GPX2-loss sensitises CRCs to ROS-inducing stimuli such as radiotherapy and chemotherapy.

Methods: Genes differentially expressed between well- and poorly differentiated CRC cell lines as well as primary and metastatic tumours were examined using RNA-sequencing data. Subsequently, isogenic, and optically barcoded GPX2-high and GPX2-low cell line systems were established to determine in vitro and in vivo sensitivity to radiotherapy and chemotherapy. Associations between GPX2-expression and clinical response to these treatments were assessed utilising in-house and publicly available cohorts.

Results: GPX2-low CRC cell lines were significantly more sensitive to clinically relevant chemotherapy in vitro, and ionising radiation both in vitro and in vivo compared to lines which retain GPX2-facilitated redox tone. Importantly, re-expression of GPX2 reduced both basal ROS levels and sensitivity to radiation and chemotherapy, whilst GPX2 deletion conferred the opposite effect, establishing a direct role for GPX2 in determining response to ROS-inducing therapeutics. Finally, retrospective analyses of an in-house, and two publicly available clinical cohorts validated that patient's with GPX2-low rectal cancers respond preferentially to radiotherapy (AUC 0.67-0.80). Additionally, compared to GPX2-high cases, GPX2-low status amongst stage III CRCs was found to be associated with superior relapse free survival benefit from adjuvant chemotherapy (HR 0.61, 95%CI 0.39-0.99, p=0.047).

Conclusion: This study identifies GPX2 expression status as a novel predictive biomarker of radiotherapy and chemotherapy response in CRCs.

Hiroki Fujimoto: Is tumor-associated fibrosis the key microenvironment for ovarian cancer progression and metastasis?

Hiroki Fujimoto[1,2], Masato Yoshihara[2], Shohei Iyoshi[2], Kazumasa Mogi[2], Emiri Miyamoto[2], Kazuhisa Kitami[3], Kaname Uno[2,4], Mai Sugiyama[5], Yoshihiro Koya[5], Yoshihiko Yamakita[5], Akihiro Nawa[5], Hiroaki Kajiyama[2], Martin K Oehler[1,6], and Carmela Ricciardelli[1]

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Abstract

Background and Aims

Tumor-associated fibrosis (TAF) represents a pathological condition characterized by the infiltration and proliferation of mesenchymal tumor cells. This study examined the role of TAF as a potential therapeutic target in the process of ovarian cancer (OC) acquiring high metastatic and malignant hallmarks.

Methods

Highly metastatic cell line, OV-90-IP4, was established by 4-times serial intraperitoneal passages in mice using serous ovarian cancer cell line, OV-90. Functional analysis of OC malignancy progression was performed in vitro and in vivo by comparing OV-90-IP4 and OV-90 cells, and the percentage of disseminated foci TAF was quantitatively analyzed by Masson-trichrome/Sirius-red staining. We further confirmed the cellular origin of TAFs using conditional knock-in mice with LoxP-stop-LoxP sequence and tdTomato inserted (WT1CreERT2/+; ROSA26fstdTomato), and examined their impact on malignant characteristics by inhibiting their invasiveness.

Results

Compared with parental OV-90 cells, 1) mesenchymal phenotype was induced in OV-90-IP4 cells, and invasive ability, proliferative capacity, and mouse seeding formation were significantly enhanced with enhanced epithelial-to-mesenchymal-transition(EMT)-associated characteristics (invasion:162%, proliferation:148%, murine peritoneal dissemination:198%), 2) TAF was significantly enhanced by Masson-trichrome staining, 3) TAF was found to be derived from EMT-transformed peritoneal mesothelial cells (MCs) using conditional knock-in mice, 4) RNA sequence analysis showed the different expressions of extracellular matrix (ECM)-associated genes, such as several types of collagen molecules. 5) Furthermore, LC-MS/MS of the xenografts' tumor indicated the tumor-stromal crosstalk causing the TAF of OV-90-IP4 cells.

Conclusion

TAF may provide a tumor-favorable microenvironment for OC progression and metastasis. Normalizing the TAF may be an effective therapeutic strategy.

ECR 3 min flash talks and interpretation from consumers

Chamikara Liyanage: Improving Immunotherapy Treatment Response In Advanced Breast Cancers

Guleria S, 1**, Liyanage C**, 1, Mangiola S, 2,3, Bell C, 1, Anderson R L, 1, Yeo B, 1,4, Vasanthakumar A, 1, Pal B, 1

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2 The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC
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4 Austin Health, Heidelberg, VIC

Abstract
Background: Immune checkpoint blockade (ICB) has shown significant success in treating melanoma and lung cancer, yet its efficacy has been limited in the treatment of early breast cancers. This could be partly due to recruitment of T regulatory cells (Tregs), which suppress the cytotoxic and anti-tumour functions of CD8+ T and natural killer (NK) cells. Hence, we hypothesise that Treg cell-mediated immunosuppression in primary tumour may promote tumour growth and metastasis.

Aims: We investigated the role of Inducible T cell co-stimulator (ICOS) receptor in Treg-mediated breast cancer growth and metastasis.

Methods: In-vivo mammary tumour models were established to evaluate the effect of loss of ICOS receptor expression and monoclonal antibody treatments blocking ICOS receptor and current ICBs. Flow cytometry and multiplex immunohistochemistry was employed to determine the affected immune cell populations and their activity in mammary tumours and metastatic sites.

Results" Systemic deletion of ICOS suppressed the Treg cell activation and proliferation followed by a significant increase in the tumour infiltrating cytotoxic CD8+ T, NK cells and their activity. Furthermore, both loss of ICOS receptor expression and anti-ICOS treatment reduced the mammary tumour growth rate in-vivo. Interestingly, the treatment substantially reduced lung metastasis formation and increased survival rates in-vivo.

Conclusion: Pharmacological blockade of ICOS receptor in combination with ICB may provide a potential therapeutic strategy to improve the metastatic-free survival of breast cancer.

Sreeja Gadipally: Identifying aggressive clones in metastatic breast cancer models

Sreeja Gadipally[1,2], Jean Berthelet[1,2], Samuel Lee[3,4], Dharmesh Bhuva[3,4,5,6], Farrah El-Saafin[1,2], Yunjian Wu[1,2], David Baloyan[1,2], Melissa Davis[3,4,5,6,7], Belinda Yeo[1,2,8], Delphine Merino[1,2,4,9]

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6. Department of Clinical Pathology, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, VIC 3052, Australia
7. Fraser Institute, University of Queensland, Woolloongabba, QLD 4102, Australia
8. Austin Health, Heidelberg, VIC 3084, Australia
9. Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia

Abstract

Aims: Identification of breast cancer clonal fitness in vivo
Investigating the molecular features of aggressive metastatic clones

Background: Breast cancer clones with distinct cellular and molecular characteristics may contribute differently to the disease progression and show variable responses to the given drugs. Some of these clones can be aggressive showing strong metastatic potential. To develop tailored treatment strategies, it is essential to understand the specific genes/pathways responsible for the aggressive behaviour of these cancer cells. We are studying this intra-tumour heterogeneity in patient samples using cellular barcoding techniques.

Methods: Human cancer cells were tagged with optical barcodes by lentiviral infection and transplanted into the mammary fat pads of immunodeficient mice. Tumours were resected and organs were collected at the experimental endpoint. Clonal fate and fitness in primary and metastatic organs were studied by flow cytometry, followed by molecular characterization of the clones by RNA sequencing.

Results: Cancer clones with variable fitness in the primary tumours were detected. Interestingly, comparing the fate of human breast cancer clones injected in multiple mice, we found that some have a higher likelihood of metastasising to specific organs than others.

Conclusion: Barcoding and tracking breast cancer clones allowed us to understand clonal dynamics during metastatic progression. Analysing the transcriptomic landscape of these clones by RNA sequencing will enable us to study the pathways associated with metastasis, aggressiveness, and clonal fitness in vital organs, thus opening a gateway to target these pathways with clinically relevant therapies.

Terrance Lam: HOXC12: The Master Regulator of β2-Adrenoceptor-Mediated Invasion in Triple Negative Breast Cancer

Terrance Lam1, Bailey Cardwell1, Bonan Liu1, Alastair C Keen1, Aeson Chang1, Erica K Sloan1,2,3, Michelle L Halls1.

1Drug Disc Biol Theme, Monash Inst Pharm Sci, Monash University, Parkville, VIC, Australia; 2Cousins Center, UCLA Semel Inst Neurosci and Human Behav and Jonsson Comprehensive Cancer Center, University of California Los Angeles, California, USA; 3Div Cancer Surgery, Peter MacCallum Cancer Centre3, East Melbourne, VIC, Australia.

Abstract

Noradrenaline released from sympathetic nerves during chronic stress accelerates cancer metastasis by activating β2-adrenoceptors (β2ARs) on tumour cells to promote invasion. We previously identified that the β2AR drives invasion via a cAMP/calcium (Ca2+) feedforward loop in the highly metastatic triple negative breast cancer (TNBC) cell line MDA-MB-231HM (Pon et al, 2016). However, it is unclear if this mechanism extends to other TNBC cell lines.

To determine whether the β2AR-cAMP-Ca2+-invasion pathway is a common feature of TNBC, cAMP accumulation, calcium mobilisation and cellular invasion were assessed in 11 TNBC cell lines. The β2-selective adrenoceptor agonist formoterol had no effect on cAMP or Ca2+ in three TNBC cell lines: HCC1937, MDA-MB-453 and MDA-MB-436. Formoterol increased cAMP and Ca2+ in six TNBC cell lines: HCC38, HCC1143, HCC1806, HCC1395, BT549, MDA-MB-468. Activation of the cAMP/Ca2+ feedforward loop in these cell lines was associated with accelerated invasion following β2AR stimulation, suggesting that cAMP and calcium signalling pathways as key mediators in regulating cellular invasion in β2AR responsive cell lines. Principal component analysis of transcriptomic and proteomic datasets revealed higher expression of the HOXC12 transcription factor in cell lines with the feedforward loop compared to those without. CRISPR-Cas9 gene knockout of HOXC12 in the feedforward loop-positive MDA-MB-468 cell line diminished β2AR-mediated calcium signalling and invasion.

These findings highlight the complexity of TNBC cell responses to β2AR stimulation. The findings identify cAMP and calcium signalling pathways as pivotal factors in mediating cellular invasion in β2AR responsive cell lines and identify HOXC12 as a key mediator of the β2AR-cAMP-calcium feedforward loop in TNBC.

Yesha Ramani: The RNA binding protein Quaking regulates prostate cancer cell plasticity by influencing widespread changes in alternative splicing.

Yesha Ramani1, Helen M. Palethorpe1, Jacqueline Chang1, Daniel P. Neumann1, Caroline A. Phillips1, Katherine A. Pillman1, John Toubia1, B. Kate Dredge1, Andrew G. Bert1, Cameron P. Bracken1,2, Luke A. Selth3, Gregory J. Goodall1,2 Brett G. Hollier4, and Philip A. Gregory1,2

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3Flinders Health and Medical Research Institute, Flinders University, Bedford Park, SA 5042, Australia.
4Institute of Health and Biomedical Innovation, Australian Prostate Cancer Research Centre - Queensland, Princess Alexandra Hospital, Queensland University of Technology, Queensland, Australia

Abstract

One of the greatest challenges in the treatment of metastatic prostate cancer (PCa) is the development of therapeutic resistance to androgen targeted therapies (ATT). Cell lineage plasticity is increasingly being implicated as a part of adaptive response to ATT, however, the mechanisms that drive this plasticity are not well understood. Our lab has previously identified an RNA binding protein, Quaking (QKI), that promotes breast cancer cell plasticity by regulating changes in alternative mRNA splicing. In PCa clinical samples, we find QKI is induced during PCa progression, increases upon ATT, and is further elevated in castrate resistant PCa (CRPC). In LNCaP cells, QKI is markedly upregulated by treatment with the ATT Enzalutamide (ENZ) and modulates ENZ-induced changes in cell plasticity and alternative splicing. Furthermore, knockout of QKI in the ENZ-resistant LNCaP derived cell line MR42D re-sensitises these cells to ENZ. In highly metastatic PC-3 cells, QKI modulates hallmark features of metastasis including changes in cell morphology, migration, and invasion with concomitant changes in alternative splicing. These studies reveal a QKI-regulated alternative splicing program influences PCa progression and the development of resistance to ATT. Overall, findings from this project will pave the way for novel alternative splicing targeting therapies to treat resistant PCa.

Michael Trpceski: Pinpointing and targeting novel drivers of pancreatic cancer progression and metastasis using TRAP-seq.

Trpceski, M.1,2, Ip, K.1,2, Chambers, C.1,2, Ritchie, S. 1,2, Reed, D.1,2, Naeini, M.1,2, Goldstein, L.1,2, Vieira, G.1,2, Weatheritt, R.1,2, Herzog, H.1,2, Murphy, K.1,2, Chtanova, T.1,2, Timpson, P.1,2, Herrmann, D.1,2.
1 The Garvan Institute of Medical Research & The Kinghorn Cancer Centre, Sydney, NSW, Australia.
2 St Vincent's Clinical School, Faculty of Medicine, University of New South Wales Sydney, Sydney, NSW, Australia.

Abstract:
Background: Pancreatic Cancer (PC) has a low 5-year survival rate of 12%, which can be attributed to its' rapid metastatic spread and resistance to chemotherapy. Therefore, there is an urgent need to identify novel candidates that become de-regulated during progression and metastasis and can be co-targeted with standard-of-care therapies to improve patient survival.

Aims: Use innovative Translating-Ribosome-Affinity-Purification followed by RNA-sequencing (TRAP-seq) to enrich for mRNAs, which are actively being translated during mutant p53-driven PC metastasis and may therefore represent valid 'druggable' targets.

Methods: Our genetically engineered mouse models closely mimic the mutational landscape, histopathology and progression of human PC. Both models are driven by an initiating KrasG12D mutation and either loss of p53 (p53 flox mouse; poorly metastatic) or a gain-of-function mutation in p53 (p53R172H; KPC mouse; highly metastatic). They also express GFP-tagged ribosomal protein Rpl10a specifically in PC cells, which can then be immunoprecipitated with associated translating mRNAs for downstream RNA-seq.

Results: We isolated primary tumours from end-stage mice of both models, as well as matched KPC metastases. All cancer cells retain Rpl10a-GFP expression and we confirmed the genomic presence of expected metastasis driver mutations. TRAP of all samples resulted in high-quality mRNA transcripts suitable for RNA-seq. This approach allowed us to assess the translatome of metastatic PC and pinpoint molecular pathways that may drive metastasis.

Conclusions: Validation of deregulated genes in our libraries of human PC samples is currently ongoing and will be followed by functional assessment using our established PC in vitro and in vivo models.

Shruti Tushar Deshpande: A novel RNA binding protein (ZCCHC24) as a regulator of cancer cell plasticity

Shruti Deshpande* [1], Daniel P. Neumann [1], Caroline A. Phillips [1], B. Kate Dredge [1], Rachael Lumb [1], Millicent GA Bennett [1], Andrew G. Bert [1], Katherine A. Pillman [1], John Toubia [1], Cameron P. Bracken [1,2], Gregory J. Goodall [1,2], Philip A. Gregory [1,2]

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Abstract

Epithelial to mesenchymal transition (EMT) plays a significant role in facilitating cancer cell invasion, tumor metastasis, and therapy resistance. In a screen for novel regulators of EMT, we uncovered an uncharacterized CCHC type Zinc finger protein (ZCCHC24) that is highly induced in mesenchymal cells and directly repressed by the epithelial-specific miR-200 family in epithelial cells. Unlike well-known EMT-driving transcription factors, ZCCHC24 is a cytoplasmic RNA binding protein (RBP) that influences cancer cell plasticity, cell migration and invasion through post transcriptional modes of action. Using CLIP-seq we identified many RNA targets of ZCCHC24, but no specific binding motif was observed. However, ZCCHC24 bound near a consensus motif for the Pumilio RBP in many cases and interacted with Pumilio suggesting it may be required for ZCCHC24's functions. Knockout of ZCCHC24 caused breast cancer cells to lose mesenchymal features including a reduction in invasive capacity, while overexpression of ZCCHC24 caused the opposite phenotypes. We propose that ZCCHC24 is a novel regulator of cancer-associated EMT and gaining an understanding of how it operates may lead to new treatment avenues for epithelial-derived cancers.

Perspective of metastasis research

Professor Robin Anderson (Co-Head, Cancer Biology and Therapy Program; Head, Metastasis Research Laboratory, Olivia Newton-John Cancer Research Institute)

One in eight Australian women will develop breast cancer during their lifetime and more than 3000 die per year due to the spread of the cancer to other parts of the body. Our research at the ONJCRI is focused on our goal of reducing deaths from breast cancer.

After initial training in agricultural science, I completed my PhD and first postdoctoral fellowship in plant biochemistry before switching to oncology for my second postdoctoral fellowship at Stanford University. Prior to joining the ONJCRI in 2016, I spent eight years at Stanford in the Department of Radiation Oncology before returning home to join the Peter MacCallum Cancer Centre.

My research is focused on understanding the genetic regulation of metastasis, primarily in breast cancer, and identifying new targets for molecular based therapy for patients with progressive disease.

My group has developed pre-clinical models of metastatic disease that we use to identify genes, both in the tumour cells and in the tumour microenvironment, that regulate the process of metastasis to specific organs, such as the bone, liver, lung and brain. The preclinical models are also used for trialling novel anti-metastatic agents that target genes found to drive metastasis.

Professor Rik Thompson (Professor of Breast Cancer Research, Queensland Institute of Technology at the Translational Research Institute)

Prof Erik (Rik) Thompson studied extracellular matrix in the rat testis for his PhD and undertook postdoctoral training in breast cancer invasion and metastasis at NIH, USA and the Lombardi Cancer Research Center, Georgetown University Medical Center, USA. He established a breast cancer Invasion and Metastasis laboratory at Georgetown before returning to St. Vincent's Institute, Melbourne in 1997 as Group Leader for Invasion and Metastasis in the Victorian Breast Cancer Research Consortium. In 2014, he joined IHBI QUT as Professor in Breast Cancer Research and Theme Leader for 'Chronic Disease and Ageing'. In 2016, he took up the role of Associate Director of IHBI at the Translational Research Institute. This role was reclassified to Research Lead, QUT@TRI in February, 2021. Rik has worked in breast cancer for >30 years, focused primarily on invasion and metastasis, building on postgraduate training in extracellular matrix (ECM) and postdoctoral interests in matrix metalloproteinases (MMPs) and epithelial mesenchymal plasticity (EMP).

Panel Discussion: Consumers, Clinicians, Researchers (Big Qs -Q&A)

Professor Daniel Brungs (Medical Oncologist, University of Wollongong)

Professor Daniel Brungs is a Staff Specialist Medical Oncologist with an interest in treating patients with gastrointestinal, lung and brain cancers.

Daniel divides his time between clinical work and as a cancer researcher at the University of Wollongong working in a translational cancer laboratory. He appointment with Illawarra Health and Medical Research Institute (IHMRI) in the Graduate Medical School at the University of Wollongong (UoW) is as a Principal Research Fellow.

Daniel is a Principal Investigator in a number of local clinical trials evaluating new anti-cancer treatments.

Daniel completed a medical science degree at the University of NSW in 2002, and his medical degree (MBBS) from the University of Sydney in 2006. While undertaking specialist training he also completed a Masters in Medicine (Clinical Epidemiology) at USyd in 2013. Most recently he has completed a PhD in Molecular Biology as the CONCERT Translational Cancer Centre Clinical Fellow at IMHRI. His PhD research investigated novel personalised therapeutic approaches to treat gastrointestinal cancer.

My aim is to incorporate the latest research into every patient's care and provide opportunities for patients to enrol in clinical trials to access new treatments.

Ms Kathryn Leaney (Executive Committee, Cancer Voices NSW)

Kathryn has been a Consumer Representative since 2013, following her own diagnosis of breast cancer. She completed the NSW Cancer Council Consumer Involvement in Research (CIR) training and joined Cancer Voices NSW in that same year. She wants to make sure that other people diagnosed with cancer do not have to go through the same experience she had.

Kathryn is an active member of the Consumer Review Panels for the National Breast Cancer Foundation (NBCF), MRFF, Cancer Institute NSW and Cancer Council NSW as well as being a member of the Consumer Advisory Panel for Maridulu Budyari Gumal (SPHERE) Cancer CAG based at the University of NSW. She is also on the Executive Committee of Cancer Voices and manages the Consumer Involvement in Research (CIR) Program, matching consumers with researchers.

Kathryn has presented at seminars and conferences on the role of the consumer in research and has facilitated several Consumer Involvement in Research workshops on behalf of SPHERE and Cancer Voices.

Kathryn has been working with a variety of research teams at several universities for more than ten years. Researchers report that Kathryn's insight into the needs of consumers is invaluable and she has made a significant contribution to the focus of their various research over this time.

Kathryn does not have a medical or scientific background but she believes that not having a scientific background is an advantage because it means that the researcher has to explain their research in simple non-

technical terms. This is particularly important when they are applying for a research grant that includes a consumer perspective.

Professor Renea Taylor (Cancer Program, Monash University)

Professor Renea Taylor, PhD, is the co-Head of the Cancer Program at Monash Biomedicine Discovery Institute and is a Lab Head within the Prostate Cancer Research Program. Renea leads a translational research program that has established and applied clinically relevant models to study prostate cancer. Her research vision is to discover new therapies for prostate cancer, especially for aggressive cases where new treatments are urgently needed. Renea is a keen advocate of consumer engagement and plays a key role in science communication, dedicating herself to prostate cancer awareness in the community.

Associate Professor Niall Corcoran (Surgeon and Research Fellow, University of Melbourne)

Associate Professor Niall Corcoran PhD FRACS(Urol) is a urologist and translational researcher based in Melbourne. He is currently Head of Urology at Western Health, and a VMO urologist at Royal Melbourne and Frankston hospitals where his main clinical focus is the management of prostate and bladder cancer. He is a principal research fellow in the Department of Surgery, University of Melbourne, where his group investigates mechanisms of progression in early prostate cancer, prognostic biomarkers and novel neoadjuvant treatment strategies. He is also the Research and Education lead in GU Oncology at the VCCC Alliance.

Mr Graeme Sissing (Cancer Research Consumer Advocate)

I am a bladder cancer survivor. My cancer experience changed my life, and I was determined to make the change a positive one. I had three major operations since 2011 and I made it my priority to learn from the experience and use it to the benefit of cancer research and future generations. I started fundraising for cancer research in 2012 and registered as a consumer advocate in 2016. My consumer involvement includes reviewing grant applications for researchers, sitting on steering committees and advisory groups eg Health Hub and patient confidentiality. I am a member of a health literacy group. I represent consumers at research retreats and student symposiums.

Selected student/ECR talks for prizes (8 min talks + 2 min Qs)

Aeson Chang: Triple negative breast cancer hijacks the sympathetic nervous system to resist chemotherapy

Aeson Chang[1], Edoardo Botteri[2], Ryan D. Gillis[1], Lukas Löfling[2], Caroline P. Le[1,3], Alexandra I. Ziegler[1], Ni-Chun Chung[1], Matthew C. Rowe[1], Stewart A. Fabb[4], Brigham Hartley[5], Cameron Nowell[1], Sasagu Kurozumi[6,7], Sara Gandini[8], Elisabetta Munzone[9], Emilia Montagna[9], Nina Eikelis[10,11], Sarah E. Phillips[10,11], Chikako Honda[7], Kei Masuda[12], Ayaka Katayama[12], Tetsunari Oyama[12], Steve W. Cole[13,14], Gavin W. Lambert[10,11], Adam K. Walker[1,15,16], Erica K. Sloan[1,14,17]*

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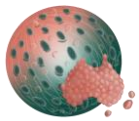
[3]Jreissati Pancreatic Centre, Epworth HealthCare, Victoria, Australia.
[4]Drug Delivery, Disposition and Dynamics Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Victoria, Australia.
[5]Insitro, South San Francisco, CA, United States of America.
[6]Department of Breast Surgery, International University of Health and Welfare, Chiba, Japan.
[7]Department of General Surgical Science, Gunma University Graduate School of Medicine, Gunma, Japan.
[8]Molecular and Pharmaco-Epidemiology Unit, Department of Experimental Oncology, European Institute of Oncology IRCCS, Milan, Italy.
[9]Division of Medical Sexology, European Institute of Oncology IRCCS, Milan, Italy.
[10]Iverson Health Innovation Research Institute and School of Health Sciences, Swinburne University of Technology, Victoria, Australia.
[11]Human Neurotransmitters Laboratory, Baker Heart & Diabetes Institute, Victoria, Australia.
[12]Department of Diagnostic Pathology, Gunma University Graduate School of Medicine, Gunma, Japan.
[13]Departments of Psychiatry & Biobehavioral Sciences and Medicine, Division of Hematology-Oncology, University of California Los Angeles, CA 90095 USA.
[14]Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and Human Behavior, and the Jonsson Comprehensive Cancer Center, University of California Los Angeles, CA 90095 USA.
[15]Neuroscience Research Australia, and the School of Psychiatry, University of New South Wales, Randwick, New South Wales, 2031, Australia.
[16]School of Psychiatry, Faculty of Medicine, The University of New South Wales, Sydney, New South Wales, 2052, Australia
[17]Division of Surgery, Peter MacCallum Cancer Centre, Melbourne Victoria 3000, Australia.

Abstract

Beta-adrenergic blockade has been associated with improved cancer survival in patients with triple-negative breast cancer (TNBC), but the mechanisms of these effects remain unclear. This project aims to investigate this by using an integrative approach of pharmacoepidemiology and animal modelling. In clinical epidemiological analyses, we identified a relationship between beta-blocker use and anthracycline chemotherapy in protecting against metastatic recurrence (hazard ratio = 0.49). We recapitulated the effect of beta-blockade on anthracycline efficacy in xenograft mouse models of TNBC. In metastatic 4T1.2 and MDA-MB-231 mouse models of TNBC, beta-blockade improved the efficacy of the anthracycline doxorubicin by reducing metastatic development by at least 3-fold. Mechanistically, we found that anthracycline chemotherapy alone, in the absence of beta-blockade, increased sympathetic nerve fiber activity and norepinephrine concentration in mammary tumors through the induction of nerve growth factor (NGF) by tumor cells. Moreover, using preclinical models and clinical samples, we found that anthracycline chemotherapy up-regulated β2-adrenergic receptor expression and amplified receptor signaling in tumor cells. Neurotoxin inhibition of sympathetic neural signaling in mammary tumors using 6-hydroxydopamine or CRISPR-induced genetic deletion of NGF or β2-adrenergic



receptor in tumor cells enhanced the therapeutic effect of anthracycline chemotherapy by reducing metastasis in xenograft mouse models. These findings reveal a neuromodulatory effect of anthracycline chemotherapy that undermines its potential therapeutic impact, which can be overcome by inhibiting β2-adrenergic signaling in the tumor microenvironment. Supplementing anthracycline chemotherapy with adjunctive β2-adrenergic antagonists represents a potential therapeutic strategy for enhancing the clinical management of TNBC.

Brooke Pereira: Temporal proteomics and advanced microscopy reveal nidogen-2 as a new stromal target in pancreatic cancer

Brooke A. Pereira1,2, Shona Ritchie1,2, Cecilia R. Chambers1,2, Katie Gordon1,2, Morghan Lucas1,2, Astrid Magenau1,2, Kendelle J. Murphy1,2, Sean Warren1,2, Max Nobis1,2, Xufeng Lin1,2, Romain Rouet1,2, Sunny Wu1,2, Julia Yin1,2, Hao-Wen Sim1,2, Lorraine Chantrill1,3, Sean Grimmond4, Anthony Gill1,5, Jeff Evans6, Leonard Goldstein1,2, Takako Sasaki7, Tri Phan1,2, Alex Swarbrick1,2, Marina Pajic1,2, Jennifer Morton8, Benjamin Parker4, David Herrmann1,2, Thomas Cox^1,2, Paul Timpson^1,2

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2. St Vincent's Clinical School, University of New South Wales, Sydney, NSW, Australia
3. Illawarra Cancer Care Centre, Wollongong Hospital, Wollongong, NSW, Australia
4. The University of Melbourne, Melbourne, VIC, Australia
5. NSW Health Pathology, Department of Anatomical Pathology, Royal North Shore Hospital, St. Leonards, NSW, Australia
6. Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, Scotland, United Kingdom
7. Department of Biochemistry, Oita University, Oita, Japan
8. Beatson Institute, Cancer Research UK, Glasgow, Scotland, United Kingdom

Abstract

Pancreatic cancer (PC) is highly lethal, with a five-year survival rate of ~11%. PC is characterised by progressive cancer-associated fibrosis. We have shown that targeting cancer fibrosis can improve chemotherapy efficacy and impair metastasis in pre-clinical models. As such, we aimed to use proteomics to dissect the matrix signatures of pancreatic tumours from the highly-metastatic KPC (Pdx1-Cre; LSL-K-rasG12D/+; LSL-p53R172H/+) and poorly-metastatic KPflC (Pdx1-Cre; LSL-K-rasG12D/+; LSL-p53fl/+) mouse models, with an aim to identify new metastatic drivers in this deadly disease.

We collected pancreatic tissue from KPflC, KPC and wildtype (WT) controls at early (~50 days), mid (~90 days) and late-stage disease (~200 days), enriched them for matrix proteins using ISDoT de-cellularisation and analysed them using data independent acquisition liquid chromatography-tandem mass spectrometry (DIA LC-MS/MS).

LC-MS/MS revealed an increased abundance of Nidogen-2 (NID2) in KPC tumours compared to KPflC. 3D organotypic matrices generated with NID2 CRISPRi CAFs had reduced fibrosis, shown via second harmonic generation (SHG) multiphoton imaging and Picrosirius Red/birefringence analysis. Organotypic invasion assays revealed that depletion of CAF NID2 significantly impeded the 3D invasion of cancer cells.

Subcutaneous and orthotopic co-seeding experiments using NID2 CRISPRi CAFs with cancer cells showed that NID2 inhibition significantly impeded tumour growth and fibrosis. Intravital imaging revealed improved vascular patency in live NID2 targeted tumours, with improved response to chemotherapy. Strikingly, in orthotopic models, mice bearing NID2 targeting had significantly reduced liver metastasis and increased survival, revealing NID2 as a new stromal target in this aggressive disease.

Charlotte Roelofs: MYC as a master regulator of dormancy in triple negative breast cancer

C. Roelofs 1,2, K. Mouchemore 1,2, A. Chakrabarti 3, R. Redvers 1,2, R. Anderson 1,2,3

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2. School of Cancer Medicine, La Trobe University, Bundoora, Vic., Australia
3. Sir Peter MacCallum Department of Oncology, the University of Melbourne, Parkville, Vic. Australia

Abstract

Introduction

Although survival rates are high, ten percent of breast cancer patients will experience relapse. Disseminated tumour cells (DTCs) can enter a dormant state, survive treatment and remain as a repository for disease recurrence. MYC, a well-known oncogene, is aberrantly regulated in many cancers including breast. The gene is involved in proliferation, tumorigenesis, and diapause. We propose MYC also regulates cancer dormancy.

Methods

We transfected an aggressive human breast cancer cell line, with naturally high MYC expression, with an inducible shRNA construct against MYC. This cell line was tested in dormancy assays and mouse models of breast cancer metastasis.

Results

MYC knockdown induced a reversible dormant phenotype in dormancy assays. Primary tumours in NSG mice displayed slower growth upon MYC suppression. Moreover, induction of MYC knockdown halted metastatic outgrowth. DTCs in lungs, livers, spine, and femurs were maintained in clusters containing few cells, while control mice displayed many and large lesions. This dormant-like state could be sustained for over 32 days after primary tumour removal. Importantly, upon MYC restoration, DTCs exited dormancy, resulting in metastatic outgrowth and recurrence. Transcriptomic analysis of dormant MYClow DTCs isolated from murine lungs and livers identified a common dormancy gene signature. In METABRIC and TCGA datasets, high expression of this signature correlated with longer relapse-free survival in breast cancer patients.

Conclusion

These data indicate MYC controls a reversible dormant phenotype in mouse models. Promisingly, expression of the MYC-driven dormancy signature correlated to patient outcome, revealing its potential as a prognostic tool.

Moganalaxmi Reckdharajkumar: Investigating the Role of Rho-ROCK Signalling in Breast Cancer Metastasis

Moganalaxmi Reckdharajkumar*1,2, Sarah T. Boyle2, Gregory J. Goodall1,2, Michael S. Samuel1,2
1University of Adelaide, 2Centre for Cancer Biology (SA Pathology and University of South Australia)

Abstract
Breast cancer is the most frequently diagnosed cancer in Australia and worldwide, accounting for over 20,000 diagnoses in Australia in 2022. Furthermore, breast cancer is currently the second leading cause of cancer-related deaths, responsible for 14% of all cancer deaths in Australian women. Most breast cancer-related deaths are due to metastasis, and the 5-year survival rate for women with metastatic breast cancer is only 20%. The Rho-ROCK (Rho-associated protein kinase) signalling axis plays an important role in several physiological and embryonic developmental processes and aberrant activation of ROCK is associated with tumour progression and metastasis in several malignancies. Our laboratory has previously established, using the PyMT mouse model of mammary cancer, that conditional activation of ROCK in mammary tumour cells triggers a paracrine signalling mechanism that recruits and reprograms fibroblasts in the tumour microenvironment to a tumour-promoting form. These reprogrammed fibroblasts upregulate their production of extracellular matrix (ECM) components to create a tumour-permissive microenvironment, thereby significantly increasing primary mammary tumour burden compared to that observed in control mice (expressing a kinase-dead (KD) version of ROCK).

To investigate the role of this pathway in metastatic disease, we tested whether ROCK activation in primary mammary tumour cells (MTCs) affected metastatic lung tumour burden in wild-type mice, injected with MTCs via the tail vein to model metastatic colonisation of the lung. Intriguingly however, we discovered that counter to our observation in the primary tumours, conditional activation of ROCK in MTCs in metastatic lung tumours suppressed fibroblast numbers despite a significant increase in the levels of tumour-promoting ECM components including collagen and periostin. We therefore hypothesise that the fibroblasts in lung metastases are a distinct population recruited by ROCK activation in tumour cells and are investigating the mechanisms underlying the observed divergent roles of ROCK in primary vs. metastatic mammary cancer.

Annabel Manoleras: Nerves talk to metastases: Characterising spatial relationships between nerves and metastatic cancer cells

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Abstract

The sympathetic nervous system (SNS) is emerging as a regulator of metastasis progression and chemotherapy efficacy. Therefore, we sought to determine the relationship between proximity to sympathetic nerves and the growth of metastatic lesions and cancer cell survival following chemotherapy. To address this, we developed a novel analysis method to define spatial interactions between sympathetic nerves and chemotherapy-surviving cancer cells in metastatic tissues.

Chemotherapy remains a first line treatment for Triple-Negative Breast Cancer (TNBC), a heterogenous cancer known to be innervated by the SNS. To visualise heterogenous subpopulations in the metastatic site, the human TNBC cell line, MDA-MB-231HM, was transduced with five Lentiviral Gene Ontology (LeGO) vectors, and 30 optically labelled clones were selected by single-cell sorting using Fluorescence-Activated Cell Sorting (FACS). Anti-Tyrosine Hydroxylase (TH) immunostaining and multispectral imaging were used to detect sympathetic nerves alongside metastasised LeGO clones in the lung and liver from doxorubicin (1 mg/kg, i.v) or vehicle-treated mice. Confocal images were segmented at a single-cell resolution using Cellpose before being analysed through our in-house TH-Distance Analysis Pipeline (TDAP).

During analysis of metastases, we made the unexpected observation that doxorubicin administration increased sympathetic nerve density in liver metastases when compared to vehicle-treated mice. In ongoing work, we are now using TDAP to define how treatment impacts neural-cancer crosstalk in metastatic organs following this observed change in neural architecture. Using this technology, our overarching goal is to guide the development of novel and repurposed drugs that modulate neural signalling in the metastatic microenvironment.

Ashna A. Kumar: A “peak” into the intratumoural distribution of a novel, small-molecule anti-metastatic agent via mass spectrometry imaging in human pancreatic ductal adenocarcinoma

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Abstract
Background: Urokinase plasminogen activator (uPA) is a key mediator of extracellular matrix remodelling and tumour cell invasiveness by converting plasminogen into the broad-spectrum serine protease plasmin. In pancreatic ductal adenocarcinoma (PDAC), characterised by rapid metastatic onset, elevated uPA levels significantly correlate with poorer survival. We describe BB2-30F, a novel, small-molecule uPA-selective inhibitor, which demonstrates complete inhibition of metastasis in a PDAC orthotopic xenograft model.

Aim: We aim to (1) confirm intratumoural distribution of BB2-30F ex vivo using matrix-assisted laser/desorption ionisation – mass spectrometry imaging (MALDI-MSI); (2) validate the interaction of BB2-30F with its biological target, uPA; and (3) assess its downstream effects to support its mechanism-of-action.

Methods: For MSI, 15-µm cryosections of fresh-frozen PDAC primary tumours were coated with 2,5-dihydroxybenzoic acid matrix and analysed on an Orbitrap Elite mass-spectrometer, coupled to an intermediate-pressure MALDI-ion source. BB2-30F was assessed for its inhibitory profile against human uPA and plasmin activity in fluorogenic solution-phase assays, at the surface of human PDAC AsPC-1 cells, and in tumours ex vivo.

Results: MALDI-MSI results revealed a heterogeneously distributed signal in treated tissue consistent with protonated drug analyte [(BB2-30F)+H]+ at m/z 386.2042, confirmed by MS/MS. Furthermore, BB2-30F dose-dependently inhibited AsPC-1 cell-surface uPA (IC50=162.9 nM) and unequivocally inhibited cell-surface plasmin activity downstream via modulation of its target uPA, which was similarly demonstrated in BB2-30F-treated tumour homogenates.

Conclusion: Our findings validate BB2-30F localises within PDAC primary tumours, and confirms inhibition of uPA and uPA-mediated plasminogen activation in PDAC. This merits uPA-targeting as a therapeutic opportunity for limiting metastasis in uPA-positive tumours.