OMPRN
Ontario Molecular Pathology Research Network

Pathology Matters Meeting
October 5-6, 2017

Marriott Bloor-Yorkville, Toronto
90 Bloor St E, Toronto, ON M4W 1A7

#PathologyMatters2017

Day One: Thursday October 5, 2017

Time	TOPIC	PRESENTER	Page
09:00	*Registration and breakfast*		
09:30	Opening remarks and highlights from the Network	David LeBrun	4
09:50	Adaptive Oncology	Lincoln Stein	5
10:10	**Keynote:** Cell Context, Mutation The Microenvironment. Origins of Cancer: Pathology Matters	David Huntsman	5
11:10	*Coffee Break*		
11:40	**Image Analysis clinic:** From benchmarking quality standards to sharing pathology data: what Canadian image analysis facilities do for you	John Bartlett, Trevor McKee, Shakeel Virk, Tyna Hope	5
12:40	*Lunch*		
13:40	**CPTRG presentations:** Intro on the funding stream and first round of grants	Bryan Lo	7
13:50	**CPTRG presentation:** Proteomic biomarker discovery in clinically malignant meningiomas	Phedias Diamandis	7
14:10	**CPTRG presentation:** Ezrin as a novel biomarker for response to chemotherapy in breast cancer	Peter Greer	7
14:30	*Coffee Break*		
15:00	**Breakout Session Discussion**: Disease site groups The goal of this session is to deliver a pitch for a small project addressing an area of need which could be delivered with existing Ontario resources. Groups will be led by the following facilitators: • Urological –David Berman • Breast – John Bartlett • Hematological – David LeBrun • Lung - Ming Tsao • Gastrointestinal – Chris Howlett		8
16:00	**Breakout Session Pitch Competition and Prize** (The winner will be decided by popular vote)		8
17:00	**Drinks Reception and Pathology Club Poster session:** Featuring posters from Pathology Club members, successful grantees of the Cancer Pathology Translational Research Grants (CPTRGs) and other invited abstracts	(Please see pages 15-23 for abstracts)	9

Day Two: Friday October 6, 2017

Time	TOPIC	PRESENTER	Page
08:30	*Breakfast*		
09:00	Genomics and Big Data: Ontario Data Integration Centre	Philip Awadalla	10
09:30	Pathology Research and Cancer Care Ontario	Aaron Pollet	10
10:00	OICR Technology Platforms session: Tissue Portal>Diagnostic Development>Genomics>Informatics	Ilinca Lungu, Jane Bayani, Paul Krzyzanowski, Lars Jorgensen	11
10:30	*Coffee break*		
11:00	**Pathology Project:** Pathologic Features of Aggressive Prostate Cancer	David Berman, Michelle Downes, Paul Park	12
11:40	**OMPRN Town Hall**	David LeBrun	13
12:30	*Lunch*		
13:30	**Informatics Project:** Accurate Discrimination of 23 Major Cancer Types via Whole Genome Somatic Mutation Patterns	Wei Jiao	13
13:50	**Biospecimen clinic:** From biomarker discovery research to validation: what can Canadian Biobanks do for you?	John Bartlett, Monique Albert, Peter Watson, Shakeel Virk	13
14:50	Prognostic and Predictive Gene Expression Breast Cancer Biomarkers *(this item was taken before the biospecimen clinic)*	Dennis Sgroi	14
15:30	*Close*		

This event is an Accredited Group Learning Activity (Section 1) as defined by the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada, approved by the Canadian Association of Pathologists-Association Canadienne des pathologistes.

OMPRN Pathology Matters 2017

Upon completion of the conference, participants should be able to:

- Identify a range of molecular tests and how these can be applied to cancer pathology research
- Understand the importance of molecular pathology in its application to diagnostics and research
- Compare and select new technologies in molecular pathology available in Ontario for their own research
- Access resources and molecular pathology expertise available through the network

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Day One: Thursday October 5, 2017

Opening remarks and highlights from the Network

David LeBrun

Dr. David LeBrun is Leader of the OMPRN Steering Committee and Outreach & Awareness Lead. He obtained his M.D. from Queen's University in 1984. He completed residency in Anatomical Pathology at the University of Toronto in 1990 then moved to Stanford University where he underwent postdoctoral training in lymphoma diagnosis and, as an MRC Research Fellow, in experimental research pertaining to molecular mechanisms of leukemogenesis. He has held a faculty appointment in the Department of Pathology and Molecular Medicine at Queen's University since 1994 and holds the rank of Professor. He conducts correlative and mechanistic research on lymphoma and acute lymphoblastic leukemia. His research has been supported by the Canadian Institutes of Health Research, the National Cancer Institute of Canada, the Leukemia & Lymphoma Society of Canada, the Ontario Institute for Cancer Research and the Cancer Research Society. Dr. LeBrun serves as the senior consultant in lymph node pathology for the Southeastern Ontario region.

Adaptive Oncology

Lincoln Stein

Dr. Lincoln Stein is the Interim Scientific Director of the Ontario Institute for Cancer Research (OICR) and leads the OICR's Informatics and Bio-computing Program, which undertakes the management and analysis of large integrative cancer research projects including the International Cancer Genome Consortium (ICGC) and its Data Coordination Centre. His research focuses on using network and pathway-based analysis to identify common mechanisms in multiple cancer types and to devise prognostic and predictive signatures to aid in patient management. In addition, his group works on problems relating to the genome structure and function of the nematode Caenorhabditis elegans, a model organism that has yielded many insights into cancer.

Cell Context Mutation The Microenvironment Origins of Cancer

David Huntsman

Dr. David Huntsman is a Professor in the Departments of Pathology and Laboratory Medicine and Obstetrics and Gynaecology at The University of British Columbia (UBC) and is the Dr. Chew Wei Memorial Professor of Gynaecologic Oncology. He is a Staff Pathologist at the BC Cancer Agency (BCCA), and a Consulting Pathologist at the Vancouver General Hospital (VGH). Dr. Huntsman is currently the Director of the BC multidisciplinary ovarian cancer research team (OvCaRe), Medical Director of the Centre for Translational and Applied Genomics (CTAG) at the BCCA, and co-Director of the Genetic Pathology Evaluation Centre (GPEC) at the Jack Bell Research Centre, VGH.

Dr. Huntsman research has led to development of predictive and prognostic tissue based cancer biomarkers for ovarian cancer and a wide variety of other tumour types. His team created a blueprint for subtype specific ovarian cancer control and have been leaders in the application of novel genomics technologies to ovarian cancer. As collaboration is critical in his field, Dr. Huntsman happily leads and engages in a wide number of multidisciplinary research groups. Most recently he has been working on the creation of broad based personalized medicine initiative for British Columbia.

Image Analysis clinic: From benchmarking quality standards to sharing pathology data what Canadian image analysis facilities do for you

This session will focus on the possibilities of image analysis technology in pathology research in Canada with short presentations from image analysis facilities in Ontario and a panel discussion on how this can be applied to your research.

John Bartlett

Dr. John Bartlett is a member of the OMPRN Steering Committee and Education Lead. He is director of OICR's Transformative Pathology Program and the Ontario Tumour Bank and pursuing an integrated platform of research focused on the development of novel cancer diagnostics. He is developing new diagnostic approaches to improve patient diagnosis and treatment. By gaining an understanding of the molecular complexity of cancer, accurate diagnostic approaches can be developed to ensure that patients receive the most appropriate treatment. Novel diagnostic tools are required to accurately identify molecular sub-types of cancer and to predict the effectiveness of treatment. Dr. Bartlett has contributed to the development of translational research within multi-centre clinical trials, several of which have led to new insights into selecting appropriate therapeutics for breast cancer patients. He aims to foster research in other laboratories across the province to accelerate the discovery of novel diagnostics for cancer.

Trevor McKee

Dr. Trevor McKee has 17 years of experience in the development and application of image analysis methods for biomedical imaging, from 3D time resolved high resolution microscopy to whole slide pathology analysis. He received his Ph.D. in Biological Engineering from the Massachusetts Institute of Technology, in the laboratory of Dr. Rakesh K. Jain, where he focused on developing methods to overcome barriers to drug and gene therapeutic delivery in tumors, and pioneering the application of multiphoton imaging methods to preclinical cancer models. Dr. McKee currently manages the image analysis core facility within the STTARR Innovation Centre at University Health Network. His work at the STTARR facility involves assists academic collaborators and industry partners with preclinical imaging and development and application of image analysis capabilities. His group of programmers, technicians and students have been developing tools for "tissue cytometry", extracting quantitative image-derived metrics for cell type and biomarker content from immunohistochemical and immunofluorescence stained slides in a reproducible and robust fashion.

STTARR's Analysis Core has been working closely with physicians in the UHN Pathology department on developing new cell counting analysis tools for research, and on developing a coordinated framework for ongoing validation of staining and analysis algorithms. Dr. McKee is also involved in work in correlative pathology with CT, MRI and PET imaging, studying cancer hypoxia using PET, autoradiography and immunostaining, and development of image analysis methods for tackling Imaging Mass Cytometry, a technique developed by Fluidigm Canada Inc. for staining of 30+ markers simultaneously on one tissue section. His work at STTARR has included collaborations with Pfizer Oncology, Karyopharm, Fluidigm Canada Inc, and other industry partnered research programs to test novel drugs, imaging techniques, and biomarker analysis algorithms.

Shakeel Virk

Shakeel Virk is the Director of Operations at the Queen's Laboratory for Molecular Pathology and the Bio-repository Pathology Coordinator for the Canadian Cancer Trials Group (CCTG).

Tyna Hope

Dr. Tyna Hope leads the development of digital pathology image analysis tools within the Biomarker Imaging Research Laboratory (BIRL); PI: Dr. Martin Yaffe. She has 19 years of experience developing medical image analysis algorithms, for both in vivo and digital pathology problems. She earned PhD (EE) from Dalhousie University (Halifax, NS) and then went on to several industry positions such as Cambridge Research and Instrumentation (Woburn, Mass), Centre for Imaging Technology Commercialization (Toronto, On), and Densitas (Halifax, NS). Presently she splits her time between BIRL, Proteocyte Diagnostics, and promoting STEM to Ontario elementary students through Professional Engineers of Ontario's Engineer-in-Residence program.

Cancer Pathology Translational Research Grant (CPTRG) presentations

This session features presentations from Principal Investigators from Stream 2 of the OMPRN Cancer Pathology Translational Research Grants awarded in 2017.

Bryan Lo

Dr. Bryan Lo is the Research Lead for the Ontario Molecular Pathology Research Network (OMPRN) and the lead scientist and medical director of the Ottawa Hospital's Molecular Oncology Diagnostics Laboratory. Dr Lo previously worked in San Francisco, California where he conducted research at the biotechnology firm, Genentech. Lo received his MD-PhD from the University of Toronto, finished his residency at the Hospital for Sick Children and was a postdoctoral fellow at Yale University. Dr Lo's lab will facilitate analysis of genetic flaws inside tumor cells, which will allow the personalization of treatment for each patient's individual type of cancer. It will also give patients access to the latest experimental cancer therapies and reduce wait times for test results since tissue samples will no longer have to be sent away for testing.

Proteomic biomarker discovery in clinically malignant meningiomas

Phedias Diamandis

Dr. Diamandis is an Assistant Professor at the Department of Laboratory Medicine & Pathobiology at the University of Toronto and a Neuropathologist at University Health Network (UHN) and Princess Margaret Cancer Centre (PMCC). Dr. Diamandis' current research program focuses on strategic application of contemporary molecular profiling technologies to improve our understanding of neurobiology and neurological disorders. His most recent large scale efforts have focused on mass spectrometry based proteomic methods to profile human neural tissue and brain malignancies.

Ezrin as a novel biomarker for response to chemotherapy in breast cancer

Peter Greer

Peter Greer was trained as a biochemist/virologist (PhD, McGill, 1986) and a cancer/molecular/developmental biologist (PDF, Samuel Lunenfeld Research Institute, Toronto), before taking a faculty position in the Department of Pathology & Molecular Medicine (Queen's University, 1991). He is a full professor and Director of the P&MM Graduate Program, a Senior Scientist in the Queen's Cancer Research Institute. He has served as a reviewer and/or chair of grant review panels for MRC, CIHR, NCIC, CCSRI and CRS. His research has been funded by those agencies, the CBCF, LRF and OICR-OMPRN. His research uses transgenic and engraftment mouse models of breast cancer to explore potential therapeutic targets.

Breakout Session Discussion: Disease site groups

This session will be conducted in small groups centered on five disease sites facilitated by pathology investigators in that research field (listed below). The goal of this session is to deliver a pitch for a small project addressing an area of need with a focus on what can be delivered with existing Ontario resources.

Please check your badge for group allocation. If you have not yet been assigned a room you may choose a group.

Topics

- ***Urological*** –David Berman – **Main Room - Forest Hill Ballroom**
- ***Breast*** – John Bartlett – **Rosedale Room**
- ***Hematological –*** David LeBrun - **Main Room - Forest Hill Ballroom**
- ***Lung*** - Ming Tsao – **Summerhill Room**
- ***Gastrointestinal*** – Chris Howlett **- Main Room - Forest Hill Ballroom**

Groups will be given one hour to discuss and create a pitch for a project in their disease site. Groups will then be asked to nominate a presenter(s) to pitch the idea in no more than 8 minutes. Audience members will be asked to score pitches on the following points:

1. Potential to contribute tangibly to the field of pathological tumour diagnosis, pathological classification, or prognostic/predictive testing.
2. Scientific merit and originality of the proposed approach.

3. Potential for clinical utility with respect to the diagnosis, prevention, or treatment of cancer.
4. Potential to result in substantive interdisciplinary collaboration between Ontario cancer researchers.
5. Quality of the pitch presentation.

Prizes will be awarded to the group with the highest combined overall score. Details on how to vote will be displayed on screens during this session.

Pathology Club Poster session

This session features posters from Pathology Club members, successful grantees of the Cancer Pathology Translational Research Grants (CPTRGs) for 2016 and other invited abstracts. The poster session will be accompanied by a drinks reception to begin at 5pm with a cash bar available.

(Please see pages 15-23 for abstracts)

Day Two: Thursday October 20, 2016

Genomics and Big Data: Ontario Data Integration Centre

Philip Awadalla

Dr. Philip Awadalla, PhD, is Director of Computational Biology and Senior Investigator at the Ontario Institute for Cancer Research, Professor of Population and Medical Genomics at the University of Toronto and is a Director and Principal Investigator of the Ontario Health Study/Canadian Partnership for Tomorrow Project. He is also the Director of the Genome Canada Canadian Data Integration Centre. Dr. Awadalla was trained at the University of Edinburgh and his team focuses on the development of next-generation genomics approaches, model-based tools and population-based approaches to study mutation rates, genome biology and cancer. His team's research also focuses on systems and population genomics approaches to capture signals in population-based samples or families as well as tools to capture rare or de novo variants and pathways, potentially critical to disease phenotypes. Dr. Awadalla's main research interests include identifying genomic determinants of blood disorders and cancers, understanding mutation and recombination biology and genomic epidemiology of age-related disorders in population cohorts.

Pathology Research and Cancer Care Ontario

Aaron Pollett

Dr. Aaron Pollett is an anatomic pathologist and co-Director of the Division of Diagnostic Medical Genetics at Mount Sinai Hospital. He is an Associate Professor in the Department of Laboratory Medicine and Pathobiology at the University of Toronto and is the current Provincial Head of the Pathology and Laboratory Medicine Program at Cancer Care Ontario.

Aaron has a specialty interest in gastrointestinal pathology and pathology informatics, particularly in the area of colorectal cancer and biomarker implementation. He is the review pathologist for the Familial Gastrointestinal Cancer Registry (FGICR) and the Ontario Familial Colon Cancer Registry (OFCCR), tumour-based registries constructed to analyze the genetic basis of gastrointestinal cancers.

OICR Technology Platforms session: Tissue Portal>Diagnostic Development>Genomics>Informatics

This session will focus on the pipeline of services and resources available for tissue-based research at OICR with short presentations from Program Managers and project leaders for those services and Q&A session on how this can be applied to your research.

Ilinca Lungu

Ilinca Lungu is a Research Technician with the Diagnostic Development Program at the Ontario Institute for Cancer Research (OICR). She is the lead of the Tissue Portal which serves as a single point of contact for OICR collaborators, providing access to a number of sample processing services, including DNA and RNA extraction, laser capture microdissection, and histology.

For more information about the Tissue Portal: tissue.portal@oicr.on.ca

Jane Bayani

Dr. Jane Bayani is a Scientific Associate with the Transformative Pathology Team at the Ontario Institute for Cancer Research (OICR), mandated to translate biomarkers into clinical practice through the development and validation of modern diagnostic methods suitable for pathology laboratories and engaging with commercial partners. Her team seeks to improve the clinical management of breast cancer by reducing its overtreatment, through the identification and validation of predictive and prognostic signatures to Level II and I evidence. Through this work, they expect to identify putative targets of therapeutic intervention for those who do not benefit from current therapeutic strategies. Her team is also currently involved in the management and execution of translational pathology sub-studies in a number of national and multinational clinical trials.

Dr. Bayani is a graduate of the Department of Laboratory Medicine and Pathobiology, University of Toronto and has an extensive background in Molecular Cytogenomics.

Paul Krzyzanowski

Dr. Krzyzanowski is the Program Manager for the Genomics Program at Ontario Institute for Cancer Research (OICR), mandated to provide state-of-the-art DNA/RNA sample preparation, library construction and sequencing capabilities to support translational research. His team serves the genetic analysis needs of public and private sector clients and collaborators.

Lars Jorgensen

Lars Jorgensen is the Director of Genome Sequence Informatics at Ontario Institute for Cancer Research (OICR). His team provides tools and workflows for sequence analysis and QC, with particular expertise in a wide variety of cancer sequence data.

Pathology Project: Pathologic Features of Aggressive Prostate Cancer

This session will focus on the pathology projects included in the PRONTO project funded by Prostate Cancer Canada.

David Berman

Dr. David M Berman (Director of Queen's Cancer Research Institute, Principal Investigator, and Professor of Pathology and Molecular Medicine) gave up his role as a New York City bicycle maven to pursue MD and PhD degrees at U.T. Southwestern where he cloned the cDNA for the enzyme 5-alpha reductase and elucidated its role in prostate development. He then performed anatomic pathology and subspecialty training in urologic pathology at Johns Hopkins, followed by a postdoctoral fellowship in Molecular Biology and Genetics.

He ran a research laboratory at Johns Hopkins University for 9 years prior to moving the laboratory to Queen's University in Kingston Ontario. His research focuses on high impact biomarkers in prostate and bladder cancer. He performs diagnostic surgical pathology one week per month and devotes the rest of his professional efforts to research.

Michelle Downes

Dr. Downes graduated from the Royal College of Surgeons in Ireland medical school with first class honours and undertook training in surgery leading to her Membership of the Royal College of Surgeons (MRCSI). Following this she completed a two year research fellowship in prostate cancer at University College Dublin and was awarded her Doctor of Medicine (MD) by thesis. She trained in Anatomic Pathology in Dublin, Ireland and then completed a clinical fellowship in genitourinary pathology at the University Health Network, Toronto during which time she was awarded FRCPC. She practices as a genitourinary pathologist at Sunnybrook Health Sciences Centre in Toronto. She is an assistant professor in Laboratory Medicine and Pathobiology at the University of Toronto. Her research and publications focus on prostate and bladder malignancy. She is a peer reviewer for Histopathology and Journal of Clinical Pathology and is an editorial board member of Human Pathology: Case Reports.

Paul Park

Dr. Park is a former research fellow in the Transformative Pathology Division at the OICR, and currently an Adjunct Assistant Professor in the Department of Pathology and Molecular Medicine at Queen's University. He is board certified in Anatomical Pathology and Molecular Genetic Pathology by the American Board of Pathology. His research interest

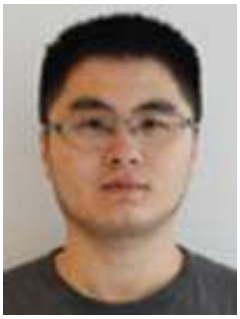
spans a wide range of the translational spectrum, from basic molecular biology to preclinical biomarker discovery/validation, and clinicopathologic correlation.

OMPRN Town Hall

Discussion lead: David LeBrun (Page 4)

This discussion will be conducted in a 'Town Hall' style, a discussion format open to everybody in a community where attendees generally present ideas and voice their opinions. Discussion will be centered on what the Ontario Molecular Pathology Research can do for its network members and the Ontario pathology research community and topics raised by network researchers ahead of the meeting.

Informatics Project: Accurate Discrimination of 23 Major Cancer Types via Whole Genome Somatic Mutation Patterns



Wei Jiao

Wei Jiao is a Research Associate in the Computational Biology team of the Stein Lab at Ontario Institute for Cancer Research (OICR).

Biospecimen clinic: From biomarker discovery research to validation what can Canadian Biobanks do for you?

This session will focus on biospecimen resources available in Ontario and Canada and how these can be leveraged for pathology researchers.

John Bartlett (Page 5)

Shakeel Virk (Page 6)

Monique Albert

Monique Albert is the Director of the Ontario Tumour Bank at OICR. She has broad experience in the health research and pharmaceutical sectors, having held a variety of management and research roles at: MaRS Excellence in Clinical Innovation and Technology Evaluation ("EXCITE") program; the STTARR Innovation Centre at the Princess Margaret Cancer Centre and the Microarray Centre (both at University Health Network); and in the corporate sector at Cangene Corporation and Affinium Pharmaceuticals.

Actively involved in the global biobanking community, she is the Director-at-Large Americas for the International Society of Biological and Environmental Repositories (ISBER). She also co-led the full Scientific Program Committee and plenary session for the ISBER international conference in May 2017. She was directly involved in the development of the Canadian Tissue Repository

Network (CTRNet) resources, has published and presented on biobanking topics on multiple occasions, and is a frequent peer reviewer for Biopreservation and Biobanking.

Peter Watson

Dr. Peter Watson is Professor of Pathology and Staff Pathologist and Senior Scientist at the BC Cancer Agency, British Columbia, Canada. He is the Director of Biobanking and Biospecimen Research Services (BBRS) which is a research infrastructure group that encompasses an active biobank and a biobanking support unit (Tumour Tissue Repository (BCCA-TTR) at the BC Cancer Agency and Office of Biobank Education and Research (UBC-OBER) at the Department of Pathology, University of British Columbia). He also leads and co-leads provincial and national efforts to foster biobank resources for translational research including the Canadian Tumour Repository Network CTRNet. He maintains an active clinical breast pathology practice and a research laboratory program focused on elucidating mechanisms of progression, understanding the intratumoral immune response, and identifying biomarkers to guide response to therapies in breast cancer.

Prognostic and Predictive Gene Expression Breast Cancer Biomarkers

Dennis Sgroi

Dr. Sgroi is a Professor of Pathology at Harvard Medical School, and the Executive Vice Chair of Pathology, Co-Director of Breast Pathology and a Member and Principal Investigator in the Division of Molecular Pathology Research and Center for Cancer Research at the Massachusetts General Hospital. Dr Sgroi received his medical degree from the University of Connecticut School of Medicine. After completing a residency and chief residency in anatomic pathology at Massachusetts General Hospital, Dr Sgroi completed a postdoctoral research fellowship in molecular pathology under the direction of Dr. Ivan Stamenkovic at Massachusetts General Hospital.
The overarching goals of research in the Sgroi laboratory are to develop better ways to identify patients who are at risk for the development of breast cancer and to identify those breast cancer patients who are likely to benefit from targeted drug therapies. His laboratory investigates the molecular genetic basis of human breast cancer and has utilized gene expression and proteomic technologies to better understand breast cancer progression. Dr Sgroi has shown that gene expression alterations are powerful predictors of breast cancer outcome and therapeutic response. His laboratory is currently focusing on determining the clinical utility of the Breast Cancer Index (BCI) biomarkers in estrogen receptor-positive, lymph node negative breast cancer patients, and understanding the molecular functional role of HOXB13 and IL17BR as it relates to anti-hormonal therapeutic response.

Use of quantitative second harmonic generation(SHG) microscopy to characterize stromal collagen organization in pancreatic ductal adenocarcinomas(PDACs)

Prashant Bavi, Joan Romero, Gun-Ho Jang, Stefano Serra, Julie Wilson, Steven Gallinger

1Department of Pathology, Laboratory Medicine Program, University Health Network and University of Toronto, Toronto, Ontario, Canada.
2 Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada
3 PanCuRx Translational Research Initiative, Ontario Institute for Cancer Research, Toronto, Ontario, Canada

Abstract

Background: PDAC tumours are characterized by an extensively desmoplastic stroma and this dense fibrosis accounts for up to 90% of overall tumour volume. Fibrillar collagens, the dominant extracellular matrix component of PDAC stroma, possess a unique non-centrosymmetric structure allowing them to act as a frequency doubler when interacting with multiphoton laser light. This coherent, nonabsorptive light interaction process can be exploited to obtain high-resolution, quantifiable images of discrete collagen fibers in a number of sources including two-dimensional histopathology specimens, and live animal models, without the need for exogenous staining. Using second harmonic generation imaging, a number of groups have shown that changes in collagen fiber structure and organization, specifically collagen alignment, length, and width during tumour initiation and progression have biologic consequences and correlate with clinical outcomes in a number of other solid tumour types.
Design: Collagen structure of 137 PDAC patients was analyzed using SHG microscopy from formalin-fixed paraffin embedded tissue microarrays. Using curvelet transform plus fibre extraction (CT-FIRE) algorithm, a free open-source collagen analysis program (http://loci.wisc.edu/software/ctfire), we quantified width, length, straightness, and alignment of collagen in this cohort. Following optimization, two regions of interest per core, each 1024 x 1024 pi, were analyzed. These parameters were correlated with corresponding patient data, including TNM stage, Margins, Age, Sex, OS, DFS, as well as various classifications of PDAC based on genomic and RNA-seq data, and immunologic data including $CD3^+$, $CD8^+$, and $CD8^+$ TILs from corresponding IHC TMAs. For 40 patients, we also analyzed if collagen structure was conserved across different tissue types, namely tumour, stromal, and immune-rich cores. We also assess intratumoral heterogeneity by comparing collagen structure parameters across tumour cores of the same patient, where available.
Results: The median age of patients was 66.25, with a range of 39.6-86.1 years. The majority of patients had tumours originating in the head of the pancreas (n = 90), with body (n = 14) and neck (n=14) being second most common. The median alignment, length, width, and straightness measurements were 0.5018 (1 being most aligned), 57.32, 4.667 (pixels), 0.9117 (1 being perfectly linear), respectively. The mean number of $CD8^+$ TILs, normalized to area (um^2), was 0.0538.

Further statistical associations including correlation with clinical, pathological, molecular and outcome data are being analyzed.

Conclusions: PDAC stromal morphology and topology of fibrillar collagen may be distinctly different in different molecular subtypes. Our study will provide quantitative information about fibrosis that will complement traditional histopathologic insights and can serve as a rich field for investigation into pathogenic and clinical implications of reorganized collagen as a PDAC disease marker.

An immunofluorescence biomarker multiplexing approach to study breast cancer heterogeneity and tumour microenvironment

Alison Cheung[1], Dan Wang[1], Tyna Hope[1], Kela Liu[1], Yulia Yerofeyeva[1], Sireesha Kaanumalle[2], Jessica Karp[2], Alex Corwin[2], Fiona Ginty[2], Sharon Nofech-Mozes[3] & Martin Yaffe[1]

1 Biomarker Imaging Research Lab, Sunnybrook Research Institute, Toronto, ON; 2 Biosciences, GE Global Research Center, Niskayuna, New York, USA; 3 Department of Anatomic Pathology, Sunnybrook Health Sciences Centre, Toronto, ON

Abstract

Both tumour cells and those in the tumour microenvironment play pivotal roles in promoting malignant progression and may affect response to treatment. In an attempt to better understand these factors, we are using quantitative multiplexed biomarker imaging to study molecules that are altered in these cell populations and their interrelationship. We used the Immunofluorescence Multiplexer (MxIF) developed by General Electric Global Research Center (GE GRC, Niskayuna, NY) which employs a sequential stain-image-bleach (SSB) approach. Single tissue sections of invasive breast cancer were labeled with a panel of antibodies of protein markers associated with breast cancer - Estrogen Receptor (ER), Progesterone Receptor (PgR) and Human Epidermal Growth Factor Receptor 2 (HER2/neu), proliferative marker (Ki67), cell cycle regulators p53 and p21, and markers of tumour-infiltrating lymphocytes (CD3, CD4 and CD8). Each antibody on the panel was conjugated to fluorochome Cy3 or Cy5. The signal intensity and staining patterns for each conjugated antibody on control tissue sections were compared to standard immunohistochemistry to optimize IF staining. Following individual optimization, each pair of Cy3- and Cy5- conjugated antibodies was sequentially applied to single tissue sections of invasive breast cancer. Using the software provided by GE GRC and analysis tools developed at BIRL, the expression of each protein marker, or lack thereof, on individual cancer cells or lymphocytes was quantified and its spatial location recorded. The percentages of cells expressing each biomarker or combination of markers were quantified. The frequencies at which cellular subsets expressing different biomarker signatures are localized in close proximity to each other were also quantified. These measurements provide estimates of the levels of heterogeneity within the tumour lesion and the microenvironment. Future study with a large patient cohort using tissue microarrays will provide a more in depth investigation of cellular heterogeneity in different subtypes of breast cancers.

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Prognostic significance and spatial heterogeneity of ERG and PTEN protein expression in multifocal prostate cancer

Tamara Jamaspishvili[1,2], Palak Patel[1,2], Rachel Livergant[1,2], Yi Niu[5,6], Yingwei P. Peng[3,4,5], David M. Berman[1,2]

[1]Cancer Biology & Genetics Division, Queen's Cancer Research Institute, Kingston, ON
[2]Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON
[3]Department of Public Health Sciences, Queen's University, Kingston, ON, Canada
[4]Department of Mathematics and Statistics, Queen's University, Kingston, ON, Canada
[5]Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute, Kingston, ON, Canada
[6]School of Mathematical Sciences, Dalian University of Technology, Dalian, Liaoning, China

Abstract

Objective: To study prognostic significance and spatial heterogeneity of ERG and PTEN status in early prostate cancer.
Methods: The study included 272 low and intermediate risk prostate cancer patients who underwent radical prostatectomy (RP) from 2000 to 2012 at Kingston General Hospital. All cases were represented on a tissue microarray. When present, analysis included both high and low grade cancer components from index and secondary tumour lesions.
Data and results: Consistent with previous studies, any PTEN loss (partial or complete) was significantly associated with biochemical recurrence (BCR) ($p<0.0001$), pathological stage ($p=0.011$) and Gleason score ($p=0.004$). Patients with both PTEN loss and strong ERG positivity had the shorter recurrence-free survival ($p=0.007$). PTEN loss was more frequent in index nodules (n=47, 96% vs n=7, 14%) and had higher risk of recurrence (HR=2.74, $p=0.00034$) compared to cases with PTEN loss only in secondary tumor nodules (HR=1.47, $p=0.59$). The extent of PTEN loss was significantly associated with the risk of recurrent disease (HR=1.245, $p=0.00036$). ERG expression was relatively consistent between and within tumor nodules, whereas PTEN deletion was strikingly heterogeneous across tumor foci. ERG overexpression was associated with higher Gleason scores but not with biochemical recurrence ($p=0.0003$ vs $p=0.744$).
Conclusions: When examined together, both PTEN loss and ERG expression have prognostic significance in early prostate cancer. Co-existence of both events portends the worst outcome. Unlike ERG, the high rate of inter- and intra-nodule heterogeneity of PTEN loss may make PTEN assessment more susceptible to biopsy sampling error.

The transcriptional regulator TBX3 promotes progression from non-invasive to invasive breast cancer

Milica Krstic, Matthew J. Cecchini, Carl O. Postenka, Joseph Andrews, Hon S. Leong, Muriel Brackstone, Ann F. Chambers, Alan B. Tuck
Departments of Pathology, Oncology, Surgery. Western University; The Pamela Greenaway- Kohlmeier Translational Breast Cancer Research Unit, London Regional Cancer Program

Abstract

In cell lines derived from the same breast cancer patient at different phases of progression, we have shown that transcriptional regulator TBX3 is abundant in invasive 21MT-1 cells, and minimally expressed in non-invasive 21NT cells.

There are two isoforms of TBX3, with different DNA binding domains. Overexpression of TBX3iso1 or TBX3iso2 in non-invasive 21NT cells resulted in increased survival, growth and invasiveness in vitro, with increased extravasation and invadopodia formation in the chick embryo. Through genome-wide ChIP-array/ChIP-Seq studies coupled to RNA-Seq we have shown that both TBX3 isoforms promote invasiveness through altered expression of EMT-related genes, including up-regulation of EMT-inducing transcription factor SLUG. SLUG expression is required for TBX3-induced migration and invasion of breast cancer cells. Assessing TBX3 levels in early stage breast cancer by immunohistochemistry revealed that expression was high in low-grade invasive lesions, suggesting TBX3 involvement in progression through the low-grade DCIS molecular pathway. We will continue to validate markers from our genomic studies (TBX3, SLUG, TWIST) by immunohistochemistry to assess whether they are correlated with increased risk for developing invasive cancer.

Differences between isoforms were also noted. Interestingly, only TBX3iso1 overexpressing cells exhibited increased tumorigenic potential in nude mice compared to the empty vector control. We have performed data mining and supporting functional studies that suggest this is likely due to the promotion of angiogenesis and secretion of several cancer-associated cytokines upon TBX3iso1 overexpression.

This work has the potential to identify patients with high-risk lesions, and to identify direct or indirect therapeutic targets to prevent disease progression.

Multispectral Whole-mount Slide Imaging and Colour Deconvolution

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Abstract

Brightfield multispectral imaging of 3-4 IHC stains can provide useful biomarker colocalization information in a format that is more familiar to clinician scientists. The benefit of whole-mount slides is to reduce the impact of sampling because it allows the pathologist to view the entire cross section of a tissue sample. However, most multispectral cameras have a field of view (FOV) that is much smaller than the tissue of interest. In order to capture the entire multispectral image of a complete 1x3, 2x3, 4x5 or 5x7 (inches) whole mount slide, a system was developed to utilize a commercial stage from a macroscopic system (Zeiss) and a multispectral camera (Perkin Elmer). Image acquisition methods were developed which leverage the multispectral camera's application program interface and the ability of the stage's companion software to run python. Image processing methods were developed within MATLAB to perform color deconvolution and open source software was used to stitch the individual FOV tiles into a single image of the tissue cross section. To validate the color deconvolution methods, the system has been tested with synthetic data. While we expect further revisions to improve speed, the system is ready for use on IHC projects.

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Path2MyDx: Personalized Molecular Pathology for Myeloid Cancer Diagnosis

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Abstract

Background: Myeloid cancer diagnosis (i.e. MPN, MDS, AML) has historically relied on subjective morphological criteria, laborious low-resolution cytogenetics, and a limited number of molecular-genetic tests (single-target/single-assay). The 2016-revised World Health Organization classification includes many new molecular features, leaving Ontario pathologists, clinicians, and patients with sub-standard tools for diagnosis.

Hypothesis: Targeted myeloid cancer next-generation sequencing (NGS) is an implementable, transferable strategy that can lead to earlier, personalized diagnoses and generate novel information about the clinical impact of mutations.

Objectives:

1) Implement and validate an NGS myeloid cancer assay (Path2MyDx).
2) Demonstrate clinical utility and inform research regarding myeloid cancer drivers.

Approach: With previous OICR support, successful utilization of NGS in early MDS (*in prep.*) and an MDS clinical trial (Sekeres et al., 2017, *J Clin Oncol*), we have been granted early access to a clinical DNA/RNA pan-myeloid NGS panel (herein called "MyNGS"). We will **first** validate MyNGS through retrospective analyses of 48 myeloid cancer samples, using established measures, including sensitivity, reproducibility, and external references. We will compare MyNGS somatic mutation, CNV and gene fusion calls with orthogonal NGS, Sanger sequencing, ARMS, RT-PCR and cytogenetic assays. **Second**, we will prospectively sequence 96 subjects with myeloid cancer and identify actionable variants, including those that: i) are essential to achieving/clarifying diagnosis; ii) alter clinical risk/follow-up; iii) present druggable targets; iv) affect decisions regarding hematopoietic stem cell transplants; and v) define patient categories for particular scrutiny, including clinical trial opportunities. **Third**, we have found *Tet2*-mutant myeloid leukemogenesis in mice is driven by inflammation. We will determine if *TET2*-mutant human myeloid cancers are also associated with inflammation. **Finally**, we will disseminate and transfer knowledge through our network of Ion Torrent NGS users in Ontario and lobby to fund such testing.

Anticipated Deliverables: Translate NGS knowledge into practice, impact health care delivery, and inform novel preventive measures.

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Identification of Biomarkers of Early Stage Urothelial Carcinoma Progression and Invasion

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Abstract

Urothelial carcinoma (UC) is the most common type of bladder cancer with approximately 9000 projected new diagnoses in Canada in 2017. Most patients present with papillary UC at the non-invasive (pTa) stage which can be treated with transurethral resection (TUR) and adjuvant intravesical chemotherapy. However, many patients experience multiple recurrences with approximately 25% of papillary non-invasive UC progressing to lamina propria invasion (pT1). Further progression into muscle invasive disease (pT2 or above) or the development of high risk recurrences will require radical cystectomy that introduces substantial morbidity and mortality. Although histology assessment is the current gold standard in urothelial carcinoma risk stratification, predicting progression to invasive disease and recurrence remains challenging.

Recent experimental data in mouse models described a Sonic Hedgehog (SHH)/Bone Morphogenetic Protein (BMP) signaling cascade that restrains UC invasion through adjacent stromal signaling. We identified public databases of human UC cohorts to explore this hypothesis. We analyzed RNA-seq data from 457 early stage papillary UC and identified 101 genes that were differentially expressed between pTa and pT1 tumors. Additional comprehensive pathway analysis identified eight differentially expressed genes that operate within a previously described SHH-regulated proliferation pathway.

To better understand the role of stromal signaling in early stage human UC invasion, we are currently comprehensively profiling both tumor and stroma using SP3-Clinical Tissue Proteomics to identify novel potential targets for clinical immunohistochemistry tests. This work will identify tumor and stromal protein signatures in early stage UC to validate SHH/BMP involvement in UC progression to invasion and holds the potential identify clinically useful markers to improve pathologic diagnosis and risk stratification.

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Papillary Renal Cell Carcinoma Histological, Immunophenotypical And Molecular Characterization; New Classification System With Associated Clinical Implications

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Abstract

Background:
Papillary Renal Cell Carcinoma (PRCC) has two histological subtypes. About 50% of the cases fail to meet all morphological criteria for either type, hence are best characterized as PRCC NOS. There are yet no reliable markers to resolve the PRCC NOS. That in turn reflects the dilemma of how to manage these patients.

Hypothesis:
PRCC type 1 and 2 are distinct tumors with distinct molecular biology. Hence their response to therapy and clinical management would differ. We further hypothesize that, molecular markers distinct to each subtype can be used to stratify the PRCC NOS cases, as a means to guide clinical management.

Methods/Results:
PRCC cohort of 115 cases was selected. Cases were subtyped histologically into PRCC types 1, 2 and NOS. Markers ABCC2, CA9, GATA3, SAll4, and BCL2 selected from our previous genomic analysis, were assessed by immunohistochemistry (IHC). A total of 31 cases were further selected for molecular analysis (miRNA, mRNA and CNV). Univariate and multivariate survival analysis were performed. ABCC2, CA9 and GATA3 exhibited distinct staining patterns between the two classic PRCC subtypes; and successfully classified the PRCC NOS cases. Moreover, immunomarkers revealed a third subtype of PRCC (35% of the PRCC cohort). Molecular analysis confirmed the presence of three distinct molecular signatures corresponding to the 3 subtypes. On univariate analysis DFS was significantly enhanced in the type1 versus 2& 3 (p value 0.047), which retained significance on multivariate analysis (p value 0.025, HR:6, 95% CI 1.25 to 32.2) .

Conclusions:
We propose a new classification system of PRCC integrating morphological, immunophenotypical, and molecular analysis. Our classification reveals a 3rd PRCC subtype. Molecularly PRCC3 has a distinct signature and clinically it behaves similar to PRCC type 2. The new classification stratifies PRCC patients into clinically relevant subgroups with significant implications to clinical management.

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High dose platinum induces cell death via mitotic catastrophe in high grade serous carcinoma cell lines but generates rare viable aneuploid subpopulations

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Background: Platinum chemotherapy is part of the first-line treatment for high grade serous carcinomas (HGSC) of tubal-ovarian origin. DNA damage induced by platinum is thought to result in apoptosis through p53-mediated signaling. But in the background of mutant p53 in HGSC, it less clear how DNA damage signaling results in cell death and whether alteration in the cell death pathway may promote platinum resistance. In this project, we utilize several established HGSC cell lines and performed detailed time course analysis using fluorescence imaging and cell cycle phase markers to demonstrate that mitotic catastrophe is a significant contributor of cell death by carboplatin but that rare viable aneuploid tumor cells were generated after surviving through mitotic slippage events.

Methods and Materials:
We used a high-content fluorescence microscopy platform and an image-cytometry analytical approach to assess the kinetics of the DNA damage response, cell-cycle checkpoint activity and cell death after in-vitro carboplatin treatment on several HGSC cell lines (OVCAR3, TOV3133G). In addition, time-lapse microscopy was performed to assess mitotic events after carboplatin treatment.

Results: Carboplatin treatment caused dose-dependent and time-dependent changes in the cell cycle. At low to intermediate dose and at early time points, tumor cells were arrested at G2. But at later time points and with higher dose treatment, carboplatin induces a paradoxical weakening of the G2-M checkpoint, resulting in aberrant progression of the cell cycle into mitosis. This was suggested by increase in phospho-histone H3 staining. Actual mitotic events were further demonstrated by time-lapse microscopy, showing that some of the initially G2-arrested tumor cells would progress to mitotic catastrophe and undergo mitotic cell death. In addition, comparison between a matched parental and the derived-resistant cell line (OVCAR3-parental vs. resistant) showed that the resistance may depend on the maintenance of the G2-M checkpoint following carboplatin treatment, which could be reversed by combining carboplatin with the checkpoint inhibitor AZD7762. Interestingly, a rare viable subpopulation of large aneuploid tumor cells emerged after surviving mitotic slippage events.

Conclusions: This study illustrates that cell death caused by platinum drugs may depend on mitotic catastrophe and that the G2-M checkpoint is a critical regulator of mitotic cell death. Resistance mechanisms circumventing mitotic cell death could be due to durable G2-M checkpoint or survival post mitotic slippage events. The rare platinum-induced aneuploid tumor cells may harbor the potential for regrowth, which is being currently assessed.