Welcome

A Message from Dr. Marco Marra

Continuing Terry's Legacy in Cancer Research

Thank you for attending our 4th annual TFRI-BC Node Research Day. We hope you enjoy today’s program. One of our goals for this meeting is to ensure our funded investigators have an opportunity to learn about the programs/projects in BC supported by the Terry Fox Research Institute and funded by the Terry Fox Foundation. With this in mind, we have presentations from both our translational and discovery teams here in the province, as well as from some of our Alberta colleagues.

We are very pleased that Dr. John Maris from Children’s Hospital of Philadelphia is able to join us this year as our keynote speaker. We are looking forward to his talk. We hope you will find all of the talks today relevant and valuable in providing a comprehensive snapshot of the Terry Fox research being conducted in our labs and clinics. You’ll find a breakdown of all the Terry Fox funded pan-Canadian research projects under way here at the back of this program book. We continue to be proud of the world-class and excellent work happening here and in partnership with other centres and partners across the country.

Last evening, it was my very good fortune to attend the trainee poster session for today’s meeting. Congratulations to all the trainees and presenters who participated for their interesting and thoughtful work in a range of areas. This afternoon, we’ll hear from selected trainees who will give rapid-fire presentations and wrap up our day with prizes for the best trainee posters and presentations.

I’d like to commend our TFRI Regional Advisory Committee (TFRI-RAC) for their work earlier this month in raising the profile of the importance of cancer research, and organizations like TFRI and TFF, to our elected MLAs in Victoria. Led by RAC chair Dr. Simon Sutcliffe, the committee – Mr. Paul Balfour, Ms. Alice Virani, Dr. Nadine Caron, and Dr. Peter Chow-White – held an engaging and enlightening meeting with 36 MLAs, including 9 ministers, to discuss how research is transforming medicine, especially in oncology. TFRI president Dr. Victor Ling and I also participated, along with Dr. Janessa Laskin and other Institute colleagues.

On behalf of our funded research community, I’d like to thank The Terry Fox Foundation for their continued investment in cancer research in BC and the Terry Fox Research Institute for their confidence in our individual and collective excellence. By continuing to work together, we will achieve Terry’s dream of ending cancer.

We hope today’s program will inspire each of you in new and productive ways. Thank you for coming.

Welcome

The Terry Fox New Frontiers Program Project

Early Detection of Lung Cancer – Molecular Correlates of Treatment Failure

Agenda

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<td>John Maris: Challenges and opportunities towards genomic biomarker-driven treatment of childhood cancer</td>
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<td>2:40 – 4:00pm</td>
<td>Panel discussion: Genomics incidental findings (GIFs) in the context of personalized medicine How do we manage GIFs in BC’s health care system?</td>
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<td>4:20 – 4:30pm</td>
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Statement on Respect for Confidentiality of Unpublished Material:

The Institute has invited everyone attending this meeting because of their contribution or potential for contribution, to the work of our research community. In building our community, we are committed to respecting the confidentiality of ideas and data that are unpublished at this meeting.

We request and require that all registrants refrain from recording such confidential information, and do not discuss such information with colleagues outside of this meeting. It is in this way that we will collectively build the trust and respect that is necessary for effective collaborations.

We appreciate your respect for and compliance with this important request.
Speakers

John M. Maris, MD
Dr. John Maris is D’Angelo Professor of Pediatrics in the Perelman School of Medicine at the University of Pennsylvania (Penn). He currently serves as Director of the Center for Childhood Cancer Research at the Children’s Hospital of Philadelphia (CHOP). Dr. Maris completed his medical degree at the Penn, and all of his post doctoral training in pediatric hematology/oncology and genetics was performed at Penn and CHOP.

Dr. Maris has published over 230 peer-reviewed manuscripts and dozens of book chapters and review articles. He is recognized as a leading expert in the field of pediatric oncology and serves on several Scientific Advisory Boards and Committees, including for the National Cancer Institute, the American Association for Cancer Research, Genome Canada and many private foundations.

Dr. Maris’ group has discovered the majority of the genes that influence susceptibility to human neuroblastoma using both traditional family-based linkage approaches and genome wide association approaches. In parallel, his group has identified many of the oncogenic drivers of the disease. Together, this work has resulted in the implementation of new genomic biomarkers of outcome that are now routinely used in the clinic, and several early phase clinical trials of new targeted therapeutics. Dr. Maris has led large collaborative research efforts focused on translational oncology, most recently a genomic dissection of over 300 high-risk neuroblastoma using next generation sequencing technologies, resulting in an unprecedented detailed characterization of the hereditary and somatic neuroblastoma genomes. Finally, Dr. Maris and a large international team were recently awarded a Stand Up To Cancer-St. Baldrick’s Foundation grant to bring the fields of genomics and immunotherapy together to combat childhood cancers. Dr. Maris serves as the Dream Team leader.

Dr. Maris has received several prestigious awards including election into the American Society of Clinical Investigation, the Oski award for outstanding pediatric oncologists, and the Berwick award at Penn for melding basic and clinical teaching, and the William Oski Patient Oriented Research Award at Penn.

Randy D Gascoyne, MD, FRCP
Randy D Gascoyne is a Clinical Professor of Pathology at the University of British Columbia (UBC), a Hematopathologist at the BC Cancer Agency (BCCA) and a Distinguished Scientist at the BC Cancer Research Centre in Vancouver, Canada.

Dr. Gascoyne obtained his BSc and MD degrees at UBC, completed an Internship at St. Paul’s Hospital in Vancouver and a Residency in Hematopathology at UBC. He joined the staff of the BCCA in 1988.

Dr. Gascoyne is best known for his work investigating the pathogenesis of lymphoid cancers using genomic approaches, gene expression profiling studies and biomarker & prognostic factor development in Hodgkin lymphoma and non-Hodgkin lymphomas. His laboratory has published seminal work regarding the role of the tumour microenvironment in lymphoid cancer biology and tumors of immune privilege. Recent publications include high ranking journals such as the New England Journal of Medicine, Science, Nature, Nature Genetics, Cancer Cell and Nature Immunology. He has more than 415 peer reviewed publications, has co-authored > 430 abstracts at major meetings and has written 20 book chapters. During his tenure at BCCA he has been a principal investigator/co-investigator on research grants totaling over $94,000,000. He serves on numerous advisory boards related to lymphoma and the editorial boards of both the Journal of Clinical Oncology and Advances in Anatomic Pathology. He served as Associate Editor of Haematologica (the Journal of the European Hematology Association) from 2008 - 2012. He is the pathology co-chair of the Eastern Cooperative Oncology Group (ECOG) Lymphoma Committee of the USA, co-chairman of the Luxemburg Lymphoma Biomarker Consortium (LLEB) and an active member of the ILMPP consortium. He serves on the scientific advisory board of the Lymphoma Research Foundation in the USA. He is an active member of the International Lymphoma Study Group (ILSG). He is currently the Research Director for the Centre for Lymphoid Cancers at the BC Cancer Agency and Head of the Department of Lymphoid Cancer Research.

In 2012 Dr. Gascoyne was elected to the Royal Society of Canada and in 2013 he was elected to the Institute of Medicine of the USA. He received the 2011 BCCA William Osler Patient Oriented Research Award at Penn. Dr. Gascoyne is best known for his work investigating the pathogenesis of lymphoid cancers using genomic approaches, gene expression profiling studies and biomarker & prognostic factor development in Hodgkin lymphoma and non-Hodgkin lymphomas. His laboratory has published seminal work regarding the role of the tumour microenvironment in lymphoid cancer biology and tumors of immune privilege. Recent publications include high ranking journals such as the New England Journal of Medicine, Science, Nature, Nature Genetics, Cancer Cell and Nature Immunology. He has more than 415 peer reviewed publications, has co-authored > 430 abstracts at major meetings and has written 20 book chapters. During his tenure at BCCA he has been a principal investigator/co-investigator on research grants totaling over $94,000,000. He serves on numerous advisory boards related to lymphoma and the editorial boards of both the Journal of Clinical Oncology and Advances in Anatomic Pathology. He served as Associate Editor of Haematologica (the Journal of the European Hematology Association) from 2008 - 2012. He is the pathology co-chair of the Eastern Cooperative Oncology Group (ECOG) Lymphoma Committee of the USA, co-chairman of the Luxemburg Lymphoma Biomarker Consortium (LLEB) and an active member of the ILMPP consortium. He serves on the scientific advisory board of the Lymphoma Research Foundation in the USA. He is an active member of the International Lymphoma Study Group (ILSG). He is currently the Research Director for the Centre for Lymphoid Cancers at the BC Cancer Agency and Head of the Department of Lymphoid Cancer Research.

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Stephen Lam, MD, FRCP
Dr. Lam is Professor of Medicine at UBC in the Respiratory Division. He is currently Chair of the BCICA Lung Tumour Group and directs the MDS Fox Early Lung Cancer Detection and Translational Research Program. He is a Distinguished Scientist in the Department of Integrative Oncology at the BC Cancer Research Centre. He is the co-PI of the TFR Pan-Canadian Early Detection of Lung Cancer Study.

Livia Garzia, PhD
Dr. Livia Garzia received a degree in medical biotechnology from the University of Naples in Italy in 2002. She then went on to pursue a PhD in life sciences at the Open University in Cambridge, UK. Dr. Garzia’s research has been focused on nervous system tumours – medulloblastoma and neuroblastoma – studying altered non-coding RNAs involved in the disease pathogenesis. She has an interest in how basic research can impact the outcome of severe diseases like paediatric brain cancers, and works towards better integration between clinicians, researchers and new tools and technology to improve tumour therapies.

Michael Blough, PhD
Dr. Michael Blough currently serves as project manager on the TFR-funded brain tumour initiative, aimed at delivering new drugs to the clinic for the treatment of a lethal type of brain tumour known as glioblastoma. Dr. Blough has been in this role since the project’s inception in July, 2012. After completing a Bachelor of Science degree in biochemistry and Master’s degree in cancer biology Dr. Blough began his PhD work under the guidance of Dr. Greg Cairncross, a leading expert in the field of neuro-oncology. During his PhD, Dr. Blough focused on understanding the underlying biology of glioblastoma, as well as molecular determinants of response to treatment. As project manager he oversees operational, financial, and legal aspects of the project.

Christopher Ong, PhD
Dr. Christopher Ong is a Senior Scientist at the Vancouver Prostate Centre and is an Associate Professor in the Department of Surgery at the University of British Columbia. He is a Michael Smith Foundation for Health Research Senior Scholar. Dr. Ong’s research interest is focused on studying disease mechanisms at the molecular and cellular level. A strong underlying emphasis of his research efforts is in translational research with the aim of translating their laboratory-based discoveries into tangible clinical applications.

In particular, his research interests are primarily focused on understanding micellar mechanisms that control treatment resistance of prostate cancer. Dr. Ong believes that insights into these pathways will guide the development of new molecular targeted therapies for treating castration resistant prostate cancer.

David F. Schaeffer, MD
Dr. David F. Schaeffer is a Clinical Assistant Professor in the Department of Pathology & Laboratory Medicine at the University of British Columbia and practices as a Gastrointestinal Pathologist at Vancouver General Hospital. Dr. Schaeffer obtained his medical degree from the Johann-Goering University of Mainz, Germany. After a Residency Program in Anatomic Pathology in Vancouver he completed his Gastrointestinal Pathology Fellowship at Mount Sinai Hospital in Toronto. Dr. Schaeffer is a co-Director of the newly formed Pancreas Centre BC and also heads the Gastrointestinal Biobank (LIBB) at Vancouver General Hospital. He has an active research program focusing predominately on translational research in colonic and pancreatic cancer.
Panellists

Peter Chow-White, MA, PhD
Dr. Peter Chow-White is an Associate Professor in the School of Communication at Simon Fraser University and Faculty of Medicine at the University of British Columbia. He is also a member of the Centre for Clinical Genomics at the Genome Sciences Centre and Advisor to the Privacy Commissioner of British Columbia. In his social science research, he investigates big data, the intersection of genomic technologies and information technologies, and personalized medicine. Dr. Chow-White’s research focuses on the impacts of big data, social media, the Internet, and technology on society. He also researches emerging issues in genomics and health, biotechnology, bioethics and the impacts of new technologies on the health care system and health care delivery.

Jan Friedman, MD, PhD
Dr. Jan Friedman is a Professor of Medical Genetics at UBC. He is a clinical geneticist who holds an MD degree from Tulane University and a PhD in Genetics from the University of Washington. In the past decade, Dr. Friedman has been a pioneer in the development and application of cytogenetic and genomic tests to understand the genetic causes and clinical consequences, and improve diagnosis of intellectual disability syndromes. He is also a recognized expert in teratology, with his recent focus on determining the risk for birth defects arising from maternal antidepressant use during pregnancy which provides critical information that is essential for the development of regulatory guidelines for these medications. His research has also led to seminal advances in improving the diagnosis of neurofibromatosis (NF) by enumerating the complex and variable spectrum of its clinical features, and in formulating recommendations for genetic and clinical diagnosis that have led to enhanced standards of patient care. His key research discoveries of novel molecular and cellular hallmarks that distinguish types of neurofibromas, benign tumors that typically arise in these patients, are providing new insights into the pathogenesis and progression of NF. Dr. Friedman has won 17 awards for his research and several teaching awards. He has participated in many committees and strategic advisory groups at UBC and externally. Dr. Friedman has published more than 240 peer-reviewed papers and numerous articles, book chapters and reports. Over the past 10 years, he has given more than 60 invited presentations.

Aly Karsan, MD, FRCP(C)
Aly Karsan, MD, FRCP(C) is the Medical Director of the Cancer Genetics Laboratory and the Centre for Clinical Genomics (CCG), Pathology and Laboratory Medicine & Distinguished Scientist, Genome Sciences Centre, BC Cancer, Professor, Department of Pathology and Laboratory Medicine, University of British Columbia. Dr. Karsan is trained as a pathologist and has a major interest in developing next generation sequencing approaches for application in the clinic. This work has led to the clinical implementation of the first CAP-compliant next generation sequencing test in Canada. Dr. Karsan oversees the CCG, which is embedded within the Genome Sciences Centre and comprises pathologists and clinical and basic scientists with genetics and genomics expertise, and bioinformaticians, as well as a social scientist and health economist. He is also actively applying genomics technologies to understanding the pathogenesis of leukemias and other cancers, and has published over 100 peer-reviewed papers.

Janessa Laskin, BSc, MD, FRCP(C)
Janessa Laskin is a medical oncologist at the British Columbia Cancer Agency in Vancouver, BC. She received her medical training, including medical oncology at the University of British Columbia and at the BC Cancer Agency. Following this, she completed a Thoracic Oncology Clinical Research Fellowship at Vanderbilt University Medical School in Nashville, TN. Dr. Laskin’s practice is focused on lung cancer but she also sees and treats people with cancers of the head and neck. Her research is primarily devoted to clinical trials, both within a number of cooperative groups as well as collaborative translational studies with a number of teams at the BC Cancer Research Centre.

Gillian Mitchell, MD, PhD
A/Prof Gillian Mitchell is a UK-trained doctor who undertook general physician training at the Guys’ group of hospitals in London and then Clinical Oncology training at the Royal Marsden Hospital, London. In 1998 she commenced a PhD in Cancer Genetics as the Institute of Cancer Research in London. A/Prof Mitchell moved to Melbourne in 2002 where she completed her oncology and cancer genetics training at the Peter MacCallum Cancer Centre. In 2005 she was appointed as the Director of the Familial Cancer Centre at Peter Mac. Since then she has developed the clinical familial cancer service into one of the leading services in Australia. A/Prof Mitchell moved to Canada in early 2014 and has been appointed as the medical director of the Hereditary Cancer Program at the BC Cancer Agency and a medical oncologist.

Her research interests include early stage trials of targeted agents in genetically predisposed cancer patients as well as studies to improve personal cancer risk prediction, targeted cancer screening programs and the integration of new genetic technology into clinical practice. In addition to clinical and research roles, A/Prof Mitchell has held positions on a number of Australian national clinical guideline committees and was the Chair of the Familial Cancer Groups of the Clinical Oncology Society of Australia and the Cancer Council Victoria.

Alice Virani, MA(Oxon), MS, MPH, PhD
Alice Virani is a clinical ethicist at the Provincial Health Service Authority and based at Children’s and Women’s Hospital in Vancouver. She also serves as the Ethicist on the Children’s and Women’s Research Ethics Board. Before moving into ethics, Alice was a genetic counselor at Columbia Presbyterian Hospital in New York. She has a Masters in Human Sciences from Oxford University, a masters in Genetic Counseling from Sarah Lawrence College, a Masters in Public Health from Columbia University, and a PhD in Genetics and Ethics from UBC. Her research interests relate to the many ethical issues inherent within clinical practice and research in genetic and genomic medicine.
John Babcock
John Babcock is Vice President of Biologics at the Centre for Drug Research and Development and is an Adjunct Professor of Molecular Biology and Biochemistry at Simon Fraser University. For over 20 years, John has made significant contributions to the international biotechnology industry. While working at Amgen, Agene and ImCloneics and through collaborations with AstraZeneca, Pfizer, Abbott, Abberton BioScience, Millennium, CellTech, Curagen, Ceva, Agenseys, Procemics and The Scopes Research Institute, he has participated in the development of over 100 therapeutic antibody programs. Six therapeutic antibodies he and his colleagues developed are now in the clinic, including three antibody-drug conjugates. At CDRI, he is responsible for establishing new antibody generation, screening, and drug conjugate platforms. He oversees Biologics pre-clinical development and is responsible for forming collaborations with academic researchers and biotech companies in BC, across Canada and internationally. In recognition of his contributions, John won the 2005 LifeSciences BC “Innovation and Achievement” Award.

Inanc Birol, PhD
Dr. Birol is a Senior Scientist at British Columbia Cancer Agency, Genome Sciences Centre (GSC), an Associate Professor of Medical Genetics at University of British Columbia, and an Adjunct Professor of Computing Science at Simon Fraser University. He heads the Bioinformatics Technology Lab at the GSC, developing innovative computational solutions and analysis pipelines, and their routine application in genomics studies. Among the technologies he developed is a scalable de novo assembly tool, AllBySS, which uses data from next generation sequencing platforms to assemble genomes. Using AllBySS, his team was the first to assemble a human genome using short reads. Today, it is one of the most popular bioinformatics tools, its download site returning a first page hit in a Google search for the English words ‘abyss’.

Connie J. Eaves, PhD, FRSc(C)
Dr. Eaves holds a BA in Biology and Chemistry and MSc in Genetics from Queen’s University, Kingston, Ontario and a PhD in Immunology from the University of Manchester in the UK. Following post-doctoral training in experimental hematology at the Ontario Cancer Institute under Dr. James F. I, she joined the faculty of the BC Cancer Agency and UBC. In 1981, she and her husband co-founded the Terry Fox Laboratory at the BC Cancer Agency and together built an internationally recognized research program in normal and cancer stem cell biology, leukemia and bone marrow transplantation. She has published more than 450 papers and has a long track record as a scientific leader in stem cell biology and as a conscientious mentor of trainees at all levels. She has also been a contributor to science policy and development in Canada and abroad and has received numerous national and international awards for her many accomplishments.

Cathie Garnis, PhD
Dr. Cathie Garnis is an Associate Professor and Director of Research for the Division of Otolaryngology (Department of Surgery) at the University of British Columbia. She completed her PhD from the University of British Columbia in 2005 and went on to a post-doctoral fellowship with Dr. Philip Sharp at the MIT Center for Cancer Research. Her research has focused on the molecular basis of development and progression for head and neck cancers (with a particular emphasis on oral carcinogenesis and through collaborations with Astrazeneca, Pfizer, Abbott, Abberton BioScience, Millennium, CellTech, Curagen, Ceva, Agenseys, Procemics and The Scopes Research Institute, he has participated in the development of over 100 therapeutic antibody programs. Six therapeutic antibodies he and his colleagues developed are now in the clinic, including three antibody-drug conjugates. At CDRI, he is responsible for establishing new antibody generation, screening, and drug conjugate platforms. He oversees Biologics pre-clinical development and is responsible for forming collaborations with academic researchers and biotech companies in BC, across Canada and internationally. In recognition of his contributions, John won the 2005 LifeSciences BC “Innovation and Achievement” Award.

Brad Nelson, PhD
Dr. Nelson is a native of Vancouver BC. He received a B.Sc. from the University of British Columbia in 1987 and a Ph.D. from the University of California at Berkeley in 1991. He completed postdoctoral training and held faculty positions at the Fred Hutchinson Cancer Research Center and University of Washington in Seattle, before moving his lab to the Benaroya Research Institute in Seattle in 1997. In 2003, he became the founding Director of the BC Cancer Agency’s Doisy Research Centre in Victoria BC. He is a Professor of Medical Genetics at the University of British Columbia and a Professor of Biochemistry/ Microbiology at the University of Victoria. Dr. Nelson’s lab studies the immune response to cancer, with an emphasis on ovarian, breast and lymphoid cancers. His team is developing a Phase I clinical trials program focused on personalized immunotherapies for cancer.

Tony Ng, MD, PhD
Dr. Ng is an Anatomical Pathologist at Vancouver General Hospital, and recently began an appointment as Clinical Assistant Professor in the Department of Pathology and Laboratory Medicine. He obtained his MD at UBC and completed the Anatomical Pathology residency program there as well. He also obtained a PhD under the supervision of Dr. Paul Sorensen, investigating calmodulin-related stress signaling mechanisms during tumor metastasis. He then went on to complete a fellowship program in Surgical Pathology at Stanford University. Dr. Ng’s clinical interests are in Haed and Neck Pathology and Bone and Soft Tissue Pathology. He is also involved in various research endeavours, in particular related cancer genomics: through work at the Genetic Pathology Evaluation Centre and the Personalized Oncogenomics Project. When he is away from the office and the microscope, he enjoys karate, chess and spending time with his wife and two kids.

Catherine Poh, DDS, PhD, FRCD (C), Dip. ABOMP
Dr. Poh is an Associate Professor, Faculty of Dentistry, University of British Columbia and a Clinician Scientist of the Integrative Oncology Department at the BC Cancer Agency. She is a dual certified oral pathologist in Canada and US and one of the 2 practicing Oral Mucosal/Structural Pathologists in BC. She is an active staff member of the Oral Oncology Department (BC Cancer Agency) and the Oral Mucosal Disease Program (Vancouver General Hospital). She is a Clinician Scientist of the Canadian Institute of Health Research (2007-2019) and a Scholar of the Michael Smith Foundation for Health Research (2007-2013). Dr. Poh is actively involved in developing processes by which new research information can be transferred into dental communities. Her primary research focus involves application of molecular and imaging tools for community screening, early detection, and management of cancerous and precancerous oral lesions. Her investigations also involve the impact of oral cancer screening in medically underserved communities. Currently she is the principle investigator of the COOLS trial, a Terry Fox Research Institute-funded, pan-Canadian multicentre phase III randomized controlled surgical trial to investigate the efficacy of using an optical device to control the local recurrence of early-stage oral cancer.

Christian Steinl, MD, PhD
Dr. Steinl is an Assistant Professor in the Department of Pathology, University of British Columbia, Canada, and Scientist in the Department of Lymphoid Cancer Research at the British Columbia Cancer Agency. He is holding an MD degree from the University of Muenster, Germany, and a doctorate degree in Pathology from the University of Witten-Herdecke, Germany. He is currently supervising a translational research laboratory focusing on the molecular pathogenesis of B cell lymphomas. Dr. Steinl is most known for his work on biomarkers in Hodgkin lymphoma, and the discovery and characterization of novel gene mutations in B cell lymphomas.
Peter Stirling, PhD

Dr. Stirling studied biochemistry at the University of Victoria (BSc) and Simon Fraser University (PhD). Following his education he trained as a postdoctoral fellow in cell biology and genetics at the University of California at Berkeley and the University of British Columbia, respectively. In 2014 he joined the Terry Fox Laboratory as a Scientist and the UBC Department of Medical Genetics as an Assistant Professor. His research interests are focused on gaining a fundamental understanding of genome maintenance as it pertains to the etiology of cancer, to cancer treatment, to other human diseases and to aging.

Stephen Yip, MD, PhD, FRCP

Stephen Yip is a board-certified neuropathologist clinically appointed at Vancouver General Hospital and is an assistant professor of medicine at the University of British Columbia. He is also trained in molecular genetics pathology and has an active research interest in brain tumour genomics. Stephen completed his combined M.D.-Ph.D. training at the University of British Columbia and followed by four years of neurosurgical training at VGH prior to switching to neuropathology. He obtained his FRCP certification in 2006 and then completed two years of research fellowship with Dr. David Louis at the Massachusetts General Hospital. He then underwent an additional year of molecular genetic pathology training in Boston prior to returning to Vancouver. He is interested in dissecting the molecular pathogenesis of low grade gliomas and genetic as well as functional intratumoral heterogeneity of brain tumours using next generation sequencing technology.

Amina Zoubeidi, PhD

Dr. Amina Zoubeidi is an Associate Professor in the Department of Urologic Sciences at University of British Columbia and a Senior Scientist at the Vancouver Prostate Centre, a National Centre of Excellence. Her research program aims at uncovering how androgen receptor activity controls phenotypic plasticity, which has been associated with the clinically relevant problem of drug resistance in prostate cancer (PCa). Her multifaceted program addresses the importance of various signaling pathways in this inherently heterogeneous disease. Importantly, amplification of different signaling pathways may be unique to an individual and their disease; therefore, her research program is designed to identify and understand common signaling nodes responsible for the emergence of resistance to ultimately design the best combination therapy to guide clinical decisions. As a critical tool in her arsenal to investigate the highly clinically relevant problem of treatment resistance to anti-androgens, the mainstay therapy for advanced PCa, she has developed a unique set of tumors and cell lines resistant to the newest anti-androgen, Enzalutamide. To-date, she has been successful as a young investigator, establishing national and international collaborations on research projects and grants from the public and private sector. This has resulted in over 100 published papers/abstracts, 5 patent applications, as well as funding as lead/coincipal investigator on grants that together total over $10 million which support her research for a total of 10 years. She serves on several grant panel review committees including PCa Canada, Canadian Institute of Health Research, Prostate Cancer Foundation USA and others. She is a member of the editorial board of Endocrine Related Cancer and ad hoc reviewer for numerous journals including EMBO, Oncogene, the AACR journals to name a few. She holds several awards including Terry Fox Young Investigator, Career Award Michael Smith for Health Research, The prostate Cancer Foundation, USA young investigator award, Faculty of Medicine Distinguished Award for Overall Early Career Excellence, overall excellence early career award, UBC and the research teaching award, excellence in basic science award, UBC among others.
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1. CLUSTERIN FACILITATES STRESS-INDUCED LIPIDATION OF LC3 AND AUTOPHAGOSOME BIOGENESIS TO ENHANCE CANCER CELL SURVIVAL

Zhang F.*, Kumano M.*+, Beraldi E.†, Fazli L.‡, Du C.†, Moore S.†, Sorensen P.‡, Zoubaidi A.†, GLEAVE M.E.†

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We define stress-induced adaptive survival pathways linking autophagy with the molecular chaperone clusterin (CLU) that promote acquired treatment resistance. Upon treatment-stress, CLU co-localizes with LC3 protein via an LIR-binding sequence within autophagosome membranes, functioning to facilitate LC3-Atg3 heterocomplex stability and LC3-lipidation, and thereby enhance autophagosome biogenesis and autophagy activation. Stress-induced autophagy is attenuated with CLU silencing in CLU-/- mice and human prostate cancer cells. CLU-enhanced cell survival occurs via autophagy-dependent pathways, and is attenuated following autophagy inhibition. Combining CLU inhibition with anti-cancer treatment attenuates autophagy activation, increases apoptosis, and reduces prostate cancer growth. This study defines a novel adaptor protein function for CLU under stress conditions, and highlights how co-targeting CLU and autophagy can amplify proteotoxic stress to delay cancer progression.

This work is supported by the Terry Fox Research Institute. Project title: The Terry Fox New Frontiers Program Project on Prostate Cancer Progression.

2. ELUCIDATING THE ROLE OF CLUSTERIN IN MITOSIS AND EVALUATING WEE1 COMPENSATORY MECHANISM AS A NEW TARGET TO OVERCOME TAXANE RESISTANCE AFTER CLUSTERIN KNOCKDOWN

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Clusterin (CLU) is a stress-activated molecular chaperone closely linked to treatment resistance and cancer progression. Importantly, CLU over-expression confers treatment resistance to taxanes, while CLU inhibition potentiates activity of most anti-cancer therapies in many preclinical models. Based on this data, the CLU inhibitor custirsen (OGX-011) was explored in combination with Docetaxel for men with CRPC. By contrast to pre-clinical data, this trial failed to show survival advantage in OGX-011 treated patients. These results suggest that compensatory survival mechanisms may occur during CLU inhibition in PCa cells, especially when used in combination with taxanes.

Cytotoxic chemotherapy drugs like taxanes are believed to gain selectivity by targeting cells that are in mitosis. Mitosis is a complex mechanism controlled the balance between different actors, Cdc25C and Wee1, which play antagonistic roles in mitosis progression. Our data indicates that CLU knockdown induces a constitutive activation of Cdc25C, which delays mitotic exit and hence sensitizes cancer cells to mitotic agents such taxane. However, unchecked Cdc25C activation, can lead to mitotic catastrophe and cell death unless cells upregulate protective mechanisms mediated through the cell cycle regulators Wee1 and Cdk2. In this study we show that Clusterin down regulation induces a constitutive activation of Cdc25C via the phosphatase PP2A. In response, cancer cells take advantage of the Wee1-Cdc2 feedback loop to balance Cdc25C activation and survive. Importantly, we believe identification of CLU regulated cell cycle effectors, such as PP2A and Wee1, may provide novel targets to improve the efficacy of combination CLU-taxane therapies in CRPC.

This work is supported by the Terry Fox Research Institute. Project title: The Terry Fox New Frontiers Program Project on Prostate Cancer Progression.
3. FORCED EXPRESSION OF C-MYC ENHANCES THE GROWTH OF PRIMITIVE HUMAN NORMAL AND CML HEMATOPOIETIC CELLS IN SINGLE-CELL AND BULK CULTURES

Bulaeva E., Beer P.A., EAVES C.J.

Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC.

MYC is a well-studied oncoprotein with the ability to activate transcriptional programs that affect cell growth, proliferation, and survival. In normal hematopoietic cells, MYC expression is essential for hematopoietic stem cell (HSC) self-renewal. However, MYC overexpression in cancer cells can lead to uncontrolled cell proliferation and cell death. Recent studies have shown that MYC expression can be dysregulated in human hematopoietic malignancies such as CML.

We isolated MYC-high and MYC-low transduced (as inferred from their GFP fluorescence) CD34+ cells by fluorescence-activated cell sorting (FACS) and cultured them in single-cell cultures containing 50 ng/ml SF, 20 ng/ml GM-CSF, IL-3, IL-6 and G-CSF, and 3 units/mL EPO for 12 days. The efficiency of clone formation was similar in all groups in both experiments performed, but the modal size of the colonies produced by cells with the highest MYC levels was on average ~10-fold larger than that of the controls. The size of the colonies produced by the MYC-low cells appeared intermediate. These findings suggest that the MYC activity promotes the growth of single progenitors, but speaks against a pro-apoptotic effect of MYC in this system.

These findings suggest that the presence of supra-normal levels of MYC in human CML and normal CB CD34+ cells activates pathways that significantly, rapidly and sustainably increase cell outputs from both these sources. Moreover, the levels of MYC activity when it is expressed under the control of the MN1 promoter (from our lentiviral vector) do not appear to compromise cell survival.

Keywords: c-MYC, Hematopoietic Stem Cell (HSC), Chronic Myeloid Leukemia (CML).

This work is supported by the Terry Fox Research Institute. Project title: The Terry Fox New Frontiers Program Project in Core Pathogenic Pathways in Human Leukemia (PI: Dr. R Keith Humphries). Sub-Project 1: Common Cellular and Pathway Elements in Therapy-Resistant AML (PIs: Drs. R Keith Humphries and Connie J Eaves).

4. COMPREHENSIVE MIRNA SEQUENCE ANALYSIS REVEALS SURVIVAL DIFFERENCES IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS


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Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada.

Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive disease, with 30-40% of patients failing to achieve complete responses to standard therapy. miRNAs are RNA molecules that attenuate expression of their mRNA targets. To characterize the DLBCL miRNome, we sequenced miRNAs from 92 DLBCL and 15 benign cord blood fresh frozen samples and from 140 DLBCL formalin-fixed, paraffin-embedded tissue (FFPET) samples for validation.

Results: We revealed known and novel miRNAs, 25 of which were associated with survival independently of cell-of-origin and International Prognostic Index scores, which are established indicators of outcome. Of these 25 miRNAs, 6 were significantly associated with survival in our validation cohort. Abundant expression of miR-28-5p, miR-214-5p, miR-339-3p and miR-5586-5p were associated with superior outcome, while abundant expression of miR-324-5p and NOVELM00203M were associated with inferior outcome. Comparison of DLBCL miRNA-seq expression profiles with those from other cancer types identified miRNAs that were more abundant in B-cell cancers. Unsupervised clustering of miRNAs identified 2 clusters of patients that had distinct differences in their outcomes. Our integrative miRNA and mRNA expression analysis revealed that the miRNAs that are increased in abundance in DLBCL may regulate the expression of genes involved in metabolism, cell cycle, and protein modification. Additionally, these miRNAs, including one novel miRNA, appeared to target chromatin modification genes that are frequent targets of somatic mutation in non-Hodgkin lymphomas.

Conclusion: Our comprehensive sequence analysis of the DLBCL miRNome identifies novel and prognostic miRNAs, reinforces results from previous mutational analyses, and reveals regulatory networks of significance for lymphomagenesis.

This work is supported by the Terry Fox Research Institute.
5. IDENTIFICATION OF DEK AS A POTENTIAL THERAPEUTIC TARGET FOR NEUROENDOCRINE PROSTATE CANCER

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Introduction: Neuroendocrine prostate cancer (NEPC) is an aggressive subtype of prostate cancer, exhibiting rapid progression and unresponsiveness to hormone therapy. Reliable prognostic assays and more effective treatments are critically required. However, the research of NEPC has been hampered by a lack of clinically relevant, experimental in vivo models of the disease. Recently we successfully developed a first-in-field patient tissue-derived xenograft model of complete neuroendocrine transdifferentiation from prostate adenocarcinoma. By comparing gene expression profiles of the parental adenocarcinoma line (LTL331) and the NEPC subtype (LTL331R), we identified DEK, a gene that has not been reported in prostate cancer, as a potential biomarker and target for NEPC.

Methods: DEK protein expression in patient tissue-derived xenograft models and clinical samples was assessed by immunohistochemistry. The biological function of DEK was determined by siRNA-induced reduction of DEK expression in prostate cancer cells followed by functional assays.

Results: Elevated DEK protein expression was observed in 100% clinical NEPC cases as distinct from their benign counterparts (0%), normal, naïve prostate cancer (3.1%) and castration resistant prostate cancer (29.5%). Increased DEK expression was an independent clinical risk factor and associated with shorter disease free survival in hormonal naïve prostate cancer patients. Reduction of DEK expression in PC-3 cells, a cell line with characteristic of NEPC, led to a marked reduction in cell proliferation, cell migration and invasion.

Conclusions: DEK may play an important role in the progression of prostate cancer, especially NEPC. Elevated DEK protein expression may serve as a poor prognostic factor for prostate cancer patients. Moreover, down-regulation of the DEK expression may be an effective therapeutic approach to treatment of NEPC.

Outcome/Impact: For the first time the clinical relevance and biological functions of DEK in prostate cancer were examined in this study, which suggested the DEK is potentially a valuable biomarker for predicting outcome of prostate cancer and provide a novel therapeutic target for treating NEPC.

This research is funded by the Terry Fox Research Institute, the Canadian Institutes of Health Research and Prostate Cancer Canada (MG, CC, YY).

6. LONGTERM MULTILINEAGE HUMAN HEMATOPOIETIC REPOPULATION OF UNTREATED KIT-MUTANT IMMUNODEFICIENT MICE


Background: Assessment of the growth and differentiation of human hematopoietic cells in immunodeficient mice has become vital to much basic and translational research. Historically, the primary focus has been to quantify different types of repopulating cells, based on the diversity and longevity of their clonal outputs in transplanted mice. With the development of mice that lack B, T and NK cells with normal life expectancies but differences in cellular DNA repair ability, an increasing application is to use mice repopulated with human cells to interrogate and perturb mechanisms controlling normal, genetically modified and malignant cell behavior. We have previously shown that C57Bl6 mice homozygous for the W41 mutation of c-kit have a functionally compromised hematopoietic stem cell (HSC) population. This enables transplanted syngeneic HSCTs to be detected at high frequency in these mice when they have been given sublethal irradiation. We now report the development and improved repopulation by human cord blood (CB) CD34+ cells of mice that have the same genetically determined B, T, NK immunodeficiency as NOD/Rag1-/-IL2R c-/- (NRG) into which a homozygous W41 gene has been introduced. This was achieved by crossing and backcrossing the progeny of NRG x C57Bl6-W41/W41 matings and selecting mice that were homozygous for the Sirpγ allele of the NOD mouse, the null Rag1 and null IL2Rgamma chain, genes of the NRG mouse and the W41 gene (obtained on an otherwise mixed NODxC57Bl6 background). The NRG mice was chosen because the Rag1 KO has no effect on the radiosensitivity of other tissues as is with the sod (S) gene in the NGS mouse.

Results: Initial studies showed that parental NRG mice given 900 cGy show similar repopulation by human CD34+ CB cells as NSG mice given 315 cGy, but are more robust with consistent longterm survival. We then performed a pilot experiment using the same transplant design (2x104 CD34+ CB cells/mouse) to compare chimerism obtained in NRG-W41 mice given an estimated “equivalent” radiosensitizing regimen of 150 cGy. The levels of multiple lineages of human cells measured in the BM 20 weeks post-transplant revealed these were greatly increased in the NRG-W41 mice (>95% human CD45+ cells vs 40% in NRG mice). Kinetic analysis of human cells in the blood also showed an enhanced output of human myeloid and B-lymphoid cells over time (B-fold higher in the NRG-W41 mice after 3 weeks). Particularly notable was the selectively increased (20-fold) and sustained output of human glycoprotein A+ (SPA+) erythroid cells in the NRG-W41 mice (5% human GPA+ cells in the BM of 20-week NRG-W41 mice given 150 cGy and 5x104 CD34+ CB cells/mouse vs 0.25% in the BM of the matched NRG mice given 900 cGy). We then investigated the extent of repopulation achievable in untreated NRG-W41 recipients. We therefore transplanted mice of both strains with 5x104 CD34+ CB cells each and have now followed the levels of human cells in their circulation and BM for up to 20 weeks. Human cells were barely detectable at 3 weeks post-transplant in either strain, but then in the unirradiated NRG-W41 mice only, their levels (all lineages) increased to close to those attained in NRG mice given 900 cGy.

Conclusion: NRG-W41 mice support robustly enhanced and long term generation in vivo of a wide range of human hematopoietic cell types, with high levels of chimerism achieved even in unirradiated primary recipients transplanted with relatively low numbers of human CD34+ CB cells.

This work is supported by the Terry Fox Research Institute. Project title: The Terry Fox New Frontiers Program Project in Core Pathogenic Pathways in Human Leukemia (PI: Dr. R.K. Humphries). Sub-Project 1: Common Cellular and Pathway Elements in Therapy-Resistant AML (Pis: Drs. R. K. Humphries and C. J. Eaves).
7. RNA-SEQ ANALYSIS REVEALS RECURRENT ALTERNATIVE SPLICING IN NEUROENDOCRINE PROSTATE TUMORS

Donmez N.1,2, Wyatt A.1, Brahmbatt S.1, Shukin R.1, Sahinalp C.S.1,2,3, Wang Y.1, COLLINS C.C.1,4

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Neuroendocrine cells, which comprise a minor fraction of cells in a healthy prostate, act as regulators of growth and secretory function of the prostate. A fraction of advanced-stage prostate tumors show increasing abundance of neuroendocrine cells signifying a differentiation from adenocarcinoma to Neuroendocrine Prostate Cancer (NEPC). While understanding the mechanism of this differentiation is of key significance in improving high-grade prostate cancer treatment, whole transcriptome analysis of such tumors has been limited to date. To address this problem, we have developed a highly accurate computational method to identify differential alternative splicing events between individual tumors using RNA-Seq data.

To investigate the splicing signatures of NEPC differentiated tumors, we applied our method on high coverage RNA-Seq data from xenograft tumors derived from a single patient, which include pre-castration, 8 and 12 weeks post-castration and the terminal NEPC sample. We have identified 436 differential splicing events between the terminal NEPC and the other time points. Interestingly, the 8 and 12 week time points do not exhibit the neuroendocrine splicing signatures and resemble the non-differentiated adenocarcinoma tumor in terms of splicing. On the other hand, the gene expression profiles of these samples show a gradual shift towards neuronal markers such as increased CHGA (chromogranin A) and NNAT (neuronatin) expression. This phenomenon supports the hypothesis that while gene expression show signs of NEPC differentiation relatively early on, splicing switches happen in the later stages of differentiation.

To test whether the predicted splicing events are shared among other NEPC tumors and not exhibited by other adenocarcinoma samples, we have extended our dataset with an independent NEPC xenograft tumor derived from another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coincide with the other neuroendocrine differentiated tumor, another patient’s penile metastasis and two other patient-derived adenocarcinoma xenografts. PCR validations show that both the metastasis and xenograft samples from this patient coin...
Copy number aberrations (CNAs) and chromosome instability (CIN) are hallmark features of cancer, yet the link between CNAs and CIN is not well understood. Given that CIN is a predisposing event that contributes to initiation and progression of cancer, identifying CNAs that drive CIN is essential. Even with the accelerated rates of discovery of amplified and/or over-expressed genes, in comparison to loss-of-function mutations, the biological relevance of most increased copy number aberrations remain unclear. This is mainly because amplified regions often include several candidate genes. To explore the effects of increased gene dosage on chromosome instability we performed two genome-wide screens in the budding yeast Saccharomyces cerevisiae. Our Chromosome Transition Fidelity (CTF) and a-like Faker (ALF) screens identified 262 dosage CIN (dCIN) genes, which includes 9 previously known dCIN genes such as CLB5, MPH1, SCM3 and CSE4. The dataset is enriched for genes involved in chromosome segregation, DNA replication and repair, transcription and nuclear transport, many of which are biological processes that protect genome integrity. To assess cross-species relevance we compiled a list of 168 human orthologs of yeast dCIN genes and found that 109 are amplified and/or over-expressed in cancer, of which 20 are recurrently amplified and are over-expressed.

We next sought to recapitulate the dosage CIN phenotype in human cells. Current methods for detecting CIN in human cells include flow cytometry and chromosome spreads, techniques that are not amenable to large-scale screening. We have therefore developed a fluorescence microscopy-based assay that utilizes a human artificial chromosome (HAC) to detect CIN in human cells. Pilot experiments with Cyclin D and Cyclin E over-expression in this HAC-based system are on going. CCND1 (Cyclin D1) and CCNE1 (Cyclin E1) are two positive controls identified in our cross-species analysis. Both genes are amplified in several different cancers and their over-expression is correlated to poor prognosis as well as resistance to chemotherapy drugs. aberrant regulation of Cyclin E results in chromosome instability and mammary epithelial cells. We plan to test several additional high priority candidate genes to recapitulate the dCIN phenotype in human cells.

Our genome-wide screens in yeast have generated a candidate list of dosage CIN genes in humans that can be used to examine pathways involved in genome stability. We plan to perform synthetic dosage lethality screens next to uncover genetic interactions specific to cells over-expressing dCIN genes. Our long-term goal is to use these conserved genetic interactions to identify and design chemotherapeutic agents that will specifically target cancer cells.
To determine whether ENZ resistance in CRPC is associated with the upregulation of immunotherapy targets
We show for the first time that CRPC patients progressing on ENZ (defined by PCWG2 criteria for biochemical,
Our data suggest that PEG10 could be a potential target in NEPC; further elucidating the PEG10 target
These results indicate that ENZ resistance is associated with the strong expression of anti-PD-1
The presence of immunotherapy targets, such as T cell checkpoint molecules PD-L1, PD-L2, PD-1 and CTLA-4,
Resistance to second generation androgen receptor (AR) inhibitors such as Enzalutamide (ENZ) inevitably
The treatment effects of the anti-androgen Enzalutamide (ENZ) in men with castration resistant prostate
11. ELUCIDATION OF AR IMPACT ON THE PATERNALLY EXPRESSED GENE 10 (PEG10)

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Background: Resistance to second generation androgen receptor (AR) inhibitors such as Enzalutamide (ENZ) inevitably occurs, and castration-resistant prostate cancer (CRPC) patients progress to neuroendocrine prostate cancer (NEPC) which is highly metastatic and lethal. Paternally expressed gene 10 (PEG10) is a retrotransposon-derived gene which has been reported to be up-regulated in NEPC, and understanding the mechanisms by which PEG10 is regulated and whether it plays a role in driving the Enzalutamide-resistant CRPC to NEPC would provide an important insight into the development of effective therapeutic regime.

Hypothesis: AR negatively regulates PEG10 to drive NEPC progression by transcriptional suppression, and PEG10 may drive NEPC.

Method: AR and PEG10 activity were manipulated in castration-sensitive, castration-resistant, and Enzalutamide-resistant cells using small molecule inhibitors, synthetic androgens, and differential media. The expressions of PEG10 and neuroendocrine (NE) markers were monitored through qRT-PCR and western blot. AR binding to PEG10 promoter region was confirmed through the ChIP assay. Luciferase activity was measured using the PEG10 plasmid transfection. siRNA for PEG10 was transfected and NE markers were monitored. Stable shRNA PEG10 cell-lines were challenged with androgen blockade and evaluated for the effect on NE markers.

Result: PEG10 is up-regulated after maximum androgen blockade mimicking castration and ENZ treatment. Androgen blockade induces PEG10 transcriptional activity. Activation of AR negatively regulates PEG10 expression. ENZ inhibits AR activity which allows for NE differentiation in CRPC and ENZ-resistant cell lines. Targeting PEG10 with siRNA inhibits NE markers. AR negatively regulates PEG10 expression.

Conclusion: Our data suggest that PEG10 could be a potential target in NEPC; further elucidating the PEG10 target pathway will provide strong support for the development of therapeutic target against NEPC and lead to improved survival outcomes in patients.

12. IMMUNOTHERAPY TARGETS ARE HIGHLY EXPRESSED IN ENZALUTAMIDE RESISTANT PROSTATE CANCER

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Introduction: The treatment effects of the anti-androgen Enzalutamide (ENZ) in men with castration resistant prostate cancer (CRPC) are short lived, thus CRPC remains an incurable disease. Immunotherapy may improve patient survival, however how efficacious these treatments are for CRPC, particularly those that inhibit T cell checkpoint pathways like PD-L1/PD-1, remains questionable. Indeed, the paucity of data showing PD-L1 expression in CRPC patients has made rationalizing the use of PD-1 blockade in these men difficult. However, whether patients with ENZ resistant CRPC may be a more relevant cohort to examine the efficacy of anti-PD-1 therapies remains unknown.

Objective: To determine whether ENZ resistance in CRPC is associated with the upregulation of immunotherapy targets on circulating cells in patients and an in vivo model.

Methods: The presence of immunotherapy targets, such as T cell checkpoint molecules PD-L1, PD-L2, PD-1 and CTLA4, were assessed by flow cytometry in PBMCs isolated from whole blood from metastatic CRPC patients prior to or after receiving ENZ, in PBMCs isolated from whole blood from mice bearing ENZ resistant or CRPC tumors, and on ENZ resistant and CRPC tumor cells grown in vitro and in vivo.

Results: We show for the first time that CRPC patients progressing on ENZ (defined by PCWG2 criteria for biochemical, radiographic or clinical progression) have an increased frequency of PD-L1+ dendritic cells (DC) compared to those naïve or responding to ENZ, as well as high frequencies of PD-L1+ T cells, in their blood. These data mirror our pre-clinical results, which show that ENZ resistant (ENZR) xenografts increase the frequency of PD-L1+ DCs and myeloid derived suppressor cells (MDSC) in the blood of tumor-bearing mice compared to CRPC, while decreasing the frequency of PD-L1+ cells infiltrating the tumor. Moreover, ENZR cells express higher levels of PD-L1 compared to CRPC.

Conclusions: These results indicate that ENZ resistance is associated with the strong expression of anti-PD-1 therapy targets, not only in the tumor, but also in circulating immune cells, and suggests investigating the efficacy of immunotherapies in ENZ resistant disease. Importantly this work provides impetus to further evaluate the contribution of tumor vs. immune cell PD-L1 expression in progression of CRPC to anti-androgen resistance and the utility of monitoring circulating cell PD-L1 pathway activity in CRPC patients to predict responsiveness to checkpoint immunotherapy.

This work is funded by Prostate Cancer Foundation USA, Urology Care Foundation, and Prostate Cancer Canada-Movember.
13. A NOVEL HIGH-THROUGHPUT SCREENING APPROACH FOR THE DETECTION OF CYTOTOXIC T-CELL RECEPTOR EPITOPES

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Infiltration of cytotoxic T-lymphocytes (CTLs) into solid tumor masses is a sign of a developed immune response to cancer, with higher numbers of tumor-infiltrating lymphocytes (TIL) correlating significantly with enhanced patient survival outcomes. In response to this phenomenon, expansion ex vivo of tumoricidal TIL and subsequent re-administration of expanded cells into patients has formed a promising new branch of cancer therapeutics, called adoptive immunotherapy. However, one of the largest remaining hurdles in the field of cancer immunotherapy is the lack of knowledge regarding the identity of tumor antigens eliciting CTL responses in cancer patients and, therefore, the inability to appropriately isolate and deploy specific tumor-reactive CTL cell lines.

Existing methods in use for T-cell antigen identification are limited in scalability and reliability and, therefore, require the biased selection of small candidate antigen panels to systematically pan for positive hits. We are developing and validating a novel methodology for identification of T-cell antigens capable of interrogating orders of magnitude more potential antigens than conventional methods. Our approach involves the use of a FRET active granzyme-B (GzmB)-cleavable reporter gene linked to a library of short peptide encoding sequences to be screened. This construct is delivered into seromatched antigen presenting cells (APC) by lentiviral gene transfer. Transduced target cells which receive a dose of GzmB from co-cultured CTL lines of interest can be detected via cleavage of the encoded reporter protein and isolated by FACS. The identity of recovered epitopes is then determined using next-generation sequencing. To our knowledge, this is the first system to employ target cells, rather than CTLs themselves, as a functional readout allowing for target cells to be pooled, screened, and recovered for direct identification of response-eliciting antigens.

Our results to date indicate that this sort-and-sequence approach is able to specifically distinguish the correctly targeted epitope out of a mixed cell population by recovering sequencing reads enriched with antigenic sequences relative to baseline. Ongoing experiments are being conducted to assess sensitivity by isolating sequences from endogenous cDNA libraries as well as signal-to-noise ratio by screening CTL against libraries of random background sequences seeded with positive epitope sequences.

14. A LYMPHOCYTE BASED CELL-TO-CELL THERAPEUTIC DELIVERY SYSTEM

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With their ability to sense and integrate a wide range of signals, and actuate context-dependent responses, engineered cell-based systems are promising next-generation therapeutics. Cytotoxic lymphocytes (CLs) are an ideal chassis for developing such systems for two reasons: (i) CLs possess a unique cell-to-cell molecular transfer system in the granzyme-perforin pathway, and (ii) T-cell receptors (TCRs), or chimeric antigen receptors (CARs), can endow CLs with an exquisite level of specificity in controlling activation of this pathway, and in targeting a cell population defined by its antigen profile.

We are developing a cell-to-cell therapeutic delivery system by engineering the granzyme-perforin pathway, which, unmodified, involves CL secretion of granzyme B (GzB) and perforin, followed by perforin facilitated GzB target cell entry and apoptosis induction. We are engineering CLs to transfer a GzB-load fusion protein to targeted cells, where the GzB motif of the fusion protein acts as a chaperone to ensure appropriate packaging, trafficking and delivery to the target cell.

We first showed the feasibility of this approach by demonstrating that a GzB-tomato fusion protein is transferred from NK-92MI natural killer cells to target K562 cells. We are now developing this system as a novel cancer therapeutic for apoptosis resistant tumor cells, a major challenge in cancer therapy. A common resistance mechanism is overexpression of the inhibitor of apoptosis protein XIAP, which we have shown renders target cells resistant to NK lysis. We have constructed a GzB-diphtheria (DTA) toxin fusion, and functionally verified its toxicity in HeLa cells. We are now evaluating the efficacy of GzB-DTA mediated NK cell killing of K562 cells that overexpress XIAP.

Engineering the granzyme-perforin pathway represents a completely novel approach to molecular delivery, which, when combined with CAR or TCR targeting, could pave the way to a new class of cell-based therapeutics, capable of executing complex in vivo therapeutic activity.
15. MICROFLUIDIC AND MASS CYTOMETRIC ANALYSES OF SINGLE HUMAN HEMATOPOIETIC STEM CELLS DEMONSTRATE DISTINCT PROLIFERATION AND SURVIVAL RESPONSES ACTIVATED BY DIFFERENTIALLY SIGNALING GROWTH FACTORS


Methods: HSCs were isolated from cord blood by FACS and quantified by limiting dilution analysis (LDA) for their ability to consecutively repopulate primary and secondary NOD/SCID-IL2Rγc-/- (NSG) mice for 30 weeks each time, before and after culture of the input cells for 21 days in serum-free medium containing 5 GFs (SCF, FLT3L, IL3, IL6, GCSF). HSC viability and proliferation was assessed by tracking single CD34+ cells in either multi-channel or poly(dimethylsiloxane) microfluidic chips imaged every 20-30 minutes, or 72 well Terakase plates scored visually every day. Mass cytometric analyses were performed simultaneously on multiple subsets of CD34+ cells fixed after 5-120 min of GF exposure. Results were obtained for 43 parameters from analyses of 1.6 million cells 14 experiments, 13 surface markers, 18 active signaling marks, 6 transcription factors, 2 survival marks, 3 cell cycle markers, and DNA content.

Results: LDA showed maintenance of input HSC numbers and quality in 21-day culture containing 5 GFs. Single-cell HSCs were isolated from cord blood by FACS and quantified by limiting dilution analysis (LDA) for their ability to maintain functional HSC numbers for 3 weeks in vitro. We find evidence of both rheostat-like and independent switch mechanisms controlling HSC survival/proliferation suggesting control of HSC biology can be significantly influenced by the balance of gene transcription towards an oncogenic phenotype. These altered epigenetic features may serve as promising therapeutic targets for treatment. In an attempt to identify epigenetic drugable targets, we performed drug screening using an epigenetic drug library (Cayman Chemical) in two SCCOHT cell lines and two ovarian cancer cell lines (SVOG3e and ES-2) with intact SMARCA4. We identified several HDAC inhibitors that selectively inhibited the viability of SCCOHT and ES-2 compared to that of SVOG3e and ES-2. We confirmed that SCCOHT cells were more sensitive to the treatment of SAHA, an FDA-approved HDAC inhibitor for treatment of cutaneous T cell lymphoma, with an IC50 around 300 nM, than other ovarian cell lines with SMARCA4 expression. Further, SCCOHT cells are also sensitive to several other pan-HDAC inhibitors including Quisinostat, Dacinostat and Givinostat, but not to RGFP999, a specific inhibitor of HDAC1/2, suggesting that class II HDACs are potential vulnerable targets of SCCOHT cells. Interestingly, HDAC6 expression is downregulated by SMARCA4 re-expression in BIN67 cells, indicating that SMARCA4 inactivation may promote oncogenic transformation partially through inducing the expression of HDAC6. Ongoing studies will address the detailed mechanisms underlying the hypersensitivity of SCCOHT cells to HDAC inhibitors and evaluate the efficacy of using HDAC inhibitors for the treatment of SCCOHT xenografts in mice.

This work is supported by the Terry Fox Research Institute. Project title: The Genomics of Forme Fruste Tumours: New Vistas in Cancer Biology and Management

16. TARGETING SMALL CELL CARCINOMA OF THE OVARY, HYPERCALCemic TYPE (SCCOHT) WITH HDAC INHIBITORS

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SCCOHT is a rare and deadly ovarian cancer type of young women. Although SCCOHT is often diagnosed at an early stage, the prognosis is nonetheless dismal with a 2-year survival less than 35% due to lack of effective treatment option. Recently, others and we have identified inactivating mutations of SMARCA4 in 93% (64/69) of SCCOHT along with loss of SMARCA4 protein. Unlike common malignancies, very few somatic non-silent mutations have been detected by paired exome or whole-genome sequencing analysis in SCCOHT and matched normal DNA. Therefore, SMARCA4 mutations appear to be the primary driver in SCCOHT tumorigenesis by rewiring the epigenetic dynamics and shifting the balance of gene transcription towards an oncogenic phenotype. These altered epigenetic features may serve as promising therapeutic targets for treatment. In an attempt to identify epigenetic drugable targets, we performed drug screening using an epigenetic drug library (Cayman Chemical) in two SCCOHT cell lines and two ovarian cancer cell lines (SVOG3e and ES-2) with intact SMARCA4. We identified several HDAC inhibitors that selectively inhibited the viability of SCCOHT cells (BIN67 and SCCOHT1) compared to that of SVOG3e and ES-2. We confirmed that SCCOHT cells were more sensitive to the treatment of SAHA, an FDA-approved HDAC inhibitor for treatment of cutaneous T cell lymphoma, with an IC50 around 300 nM, than other ovarian cell lines with SMARCA4 expression. Further, SCCOHT cells are also sensitive to several other pan-HDAC inhibitors including Quisinostat, Dacinostat and Givinostat, but not to RGFP999, a specific inhibitor of HDAC1/2, suggesting that class II HDACs are potential vulnerable targets of SCCOHT cells. Interestingly, HDAC6 expression is downregulated by SMARCA4 re-expression in BIN67 cells, indicating that SMARCA4 inactivation may promote oncogenic transformation partially through inducing the expression of HDAC6. Ongoing studies will address the detailed mechanisms underlying the hypersensitivity of SCCOHT cells to HDAC inhibitors and evaluate the efficacy of using HDAC inhibitors for the treatment of SCCOHT xenografts in mice.

This work is supported by the Terry Fox Research Institute. Project title: The Genomics of Forme Fruste Tumours: New Vistas in Cancer Biology and Management
17. DISPARATE RAPID EFFECTS OF FLT3-LIGAND AND GP130-ACTIVATING CYTOKINES ON PRIMITIVE SUBSETS OF CD34+ HUMAN HEMATOPOIETIC CELLS

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Objective: To investigate different Gp130-activating growth factor effects on different primitive subsets of human cord blood cells.

Methods: CD34+38- (38-) and CD34+38-45RA-90+49f+ (49f) cells were isolated by FACS, and cultured in serum-free medium containing 100 ng/mL FLT3-L + 20 ng/mL IL11 or 40 ng/mL hyper-IL6 (hyIL6). Phospho-flow was performed on CD34+ cells, fixed, and stained for surface and phospho-markers.

Results: LTC-IC frequency in initial 38- and 49f subsets was 1/20 (95%CI=1/10–1/44) and 1/3 (95%CI=1/2-1/7). 49f cells thus account for ~20% of all 38- LTC-ICs. After 4 days in the test cultures, 38- LTC-ICs were maintained in hyIL6, but decreased in IL11 (4x, p=0.009). 49f LTC-ICs were also maintained in IL11 and decreased in hyIL6 (3x, p=0.02). 38- cells produced larger clones in hyIL6 vs IL11 (largest clones >80 and 12 cells, respectively by day 10) and more 38- cells were BrdU+ after 4 days in culture in hyIL6 vs IL11 (49% with SD of 6% vs 15% with SD of 2%). In contrast, 49f cells showed little evidence of division in 10 days (largest clones in hyIL6 vs IL11 of 9 and 2 cells, respectively), despite similar BrdU+ cells by day 4 (31%, SD of 0.2% and 15%, SD of 10%, respectively). Both subsets showed stronger rapid phosphorylation of STAT3 in hyIL6 vs IL11, but activation of STAT5 was more prominent in the 49f cells.

Conclusions: Growth factors that activate the same receptor may elicit different signal strengths, timings, and biological effects on closely related primitive hematopoietic subsets.

18. SPECIFIC EXPRESSION OF AN ENDOGENOUS RETROVIRAL ASSOCIATED LONG NON-CODING RNA, EVADR, IN HUMAN ADENOCARCINOMA


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Long non-coding RNAs (lncRNAs) are mRNA-like transcripts that function at the RNA level in an array of biological processes. Compared to protein coding genes, lncRNAs are highly associated with transposable elements, particularly with endogenous retrovirus (ERV) LTRs. Since unregulated ERV expression can have potentially deleterious effects on gene expression, ERV elements are typically silenced in healthy somatic tissues, albeit with varying efficiency. Accumulating evidence suggests that in cancer, ERVs may be released from these regulatory controls, resulting in upregulated ERV-mediated transcription. The aberrant regulation of ERV LTRs, coupled with the diverse range of lncRNA functions, creates significant potential for ERV-lncRNAs to impact cancer biology. We have surveyed the aberrant expression profile of a novel ERV-lncRNA, EVADR, across 25 different tumor types, and report that it is strikingly associated with adenocarcinomas. Strong EVADR transcription was observed in 25-53% of colorectal, lung, pancreatic and stomach tumors, where it correlated with poor patient survival (Cox regression; HR = 1.47, P = 0.020). Intriguingly, EVADR is partially derived from a MER48 LTR which provides a functional promoter to activate transcription. However, while the EVADR lncRNA demonstrated high level, specific expression in adenocarcinomas, we found little evidence to support the genome-wide activation of MER48 LTRs in these same cancers. Therefore, EVADR upregulation is not a consequence of non-specific MER48 activation. In Old World Monkeys and apes, EVADR demonstrates high sequence conservation compared to most lncRNAs, whereas in the New World Monkeys, tarsier and prosimians it is conspicuously absent, implicating a role for this gene in primate development or biology. Taken together, our results describe the LTR-mediated expression of a highly conserved ERV-lncRNA in cancers of glandular origin, a finding with diagnostic, prognostic and therapeutic implications for patients suffering from adenocarcinoma.
19. BIRC6 AS A NOVEL THERAPEUTIC TARGET AGAINST CASTRATION RESISTANT PROSTATE CANCER

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**Introduction:** Treatment resistance, the major challenge in the management of advanced prostate cancer, is in part based on resistance to apoptosis. The Inhibitor of Apoptosis (IAP) family of proteins shows elevated expression in many types of cancer and is thought to play key roles in their survival and drug resistance via inhibition of apoptosis. Of the IAP family members, cIAP1, cIAP2, XIAP and survivin are known to be up-regulated in prostate cancer. Recently, we demonstrated that BIRC6, a much less studied IAP member, was also elevated in prostate cancer (PCa) and associated with castration-resistant prostate cancer (CRPC). We hypothesize that BIRC6 is functionally important in CRPC survival and targeting BIRC6 in combination with other IAPs can effectively suppress CRPC.

**Methods:** The expression of BIRC6 and its correlation with the expression of other IAP members in clinical specimen were examined by immunohistochemistry. Two Antisense Oligonucleotide (ASO) sequences that knock-down BIRC6 and an additional IAP member, namely 6w2 and 6w5, which target BIRC6 + cIAP1 and BIRC6 + survivin respectively, were designed. Effect of ASOs on proliferation, apoptosis and survival signaling in prostate cancer cells were examined by in vitro assays. Therapeutic potential of both dASOs were also studied in vivo using PC-3 xenograft mouse model.

**Results:** We showed positive correlations between BIRC6 and other reported IAP members (XIAP, survivin and cIAP1) in PCa clinical specimens. The two ASOs showed substantial inhibition of PC3 and C4-2 CRPC cells proliferation. The growth inhibition was associated with increased apoptosis, cell cycle arrest and suppression of NFkB activation. Moreover, both ASOs showed marked growth inhibition in a CRPC xenograft model without major host toxicity.

**Conclusion:** We have demonstrated that BIRC6, cIAP1 and survivin are important for CRPC cells survival. Current study demonstrated that the two BIRC6-based IAP targeting ASD candidates 6w2 and 6w5 can effectively suppress CRPC tumor growth and represent potential novel therapeutic agents against CRPC.

The project is funded by Terry Fox New Frontiers Program on Prostate Cancer Progression 2011 (M. Gleave), Project #5: The Anti apoptosis Protein, BIRC6: A Potential Therapeutic Target for Prostate Cancer (Y. Wang (PI) and M. Gleave).

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20. CARBONIC ANHYDRASE IX PROMOTES MYELOID- DERIVED SUPPRESSOR CELL RECRUITMENT AND ESTABLISHMENT OF A METASTATIC NICHE BY STIMULATING G-CSF PRODUCTION

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The recruitment of bone marrow-derived cells (BMDCs) to distant tissues prior to the arrival of disseminated tumor cells has been shown preclinically to facilitate metastasis through the establishment of metastatic niches. Primary tumor hypoxia has been demonstrated to play a pivotal role in the production of chemokines and cytokines responsible for the mobilization of these BMDCs, especially in breast cancer. Carbonic anhydrase IX (CAIX) expression is highly upregulated in hypoxic breast cancer cells through the action of Hypoxia-Inducible Factor-1 (HIF-1). Preclinical evidence has demonstrated that CAIX is required for breast tumor growth and metastasis, however, the mechanism by which CAIX exerts its prometastatic function is not well understood. Here, we show that CAIX is indispensable for the production of G-CSF by hypoxic breast cancer cells and tumors. Furthermore, we demonstrate that CAIX expression in the tumor is required for the G-CSF-driven recruitment of granulocytic myeloid-derived suppressor cells (MDSCs) to the breast cancer lung metastatic niche. We also determined that CAIX expression is required for the activation of NF-κB in hypoxic breast cancer cells and constitutive activation of the NF-κB pathway in CAIX-depleted cells restored G-CSF secretion. Together, these findings identify a novel hypoxia-induced CAIX-NF-κB-G-CSF cellular signaling axis culminating in the recruitment of granulocytic MDSCs to the breast cancer lung metastatic niche.

This work was supported by the Canadian Institutes of Health Research and the Canadian Breast Cancer Foundation. Project Title: Targeting carbonic anhydrase IX and hypoxia for the diagnosis and treatment of aggressive breast cancers (Project Leader: Shoukat Dedhar).
21. HSP 27 NEGATIVELY AFFECTS THE HIPPO PATHWAY TO REGULATE CELL SURVIVAL IN CANCER
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Introduction: Heat shock protein 27 (Hsp27) is a molecular chaperone highly and uniformly expressed in treatment resistant cancers like castrate resistant prostate cancer (CRPC). Hsp27 regulates activity of several oncogenic pathways its levels correlate with aggressive tumor behaviour, drug resistance and tumor growth. Similarly, dysregulation of the Hippo tumor suppressor pathway, which restricts organ size and cell proliferation, occurs in many types of cancers. In healthy cells, activation of the Hippo pathway results in phosphorylation and cytoplasmic retention of two transcriptional co-activators YAP and TAZ, whereas in cancer YAP and TAZ are free to translocate to the nucleus and increase cell proliferation by promoting the activities of certain transcriptional factors including TEAD1. Inactivation of the Hippo pathway correlates with poor patient outcome and progression of tumors as well as an increase in migration, invasion and metastatic potential of cancer cells. Therefore it is of great importance to establish the correlation between Hsp27 and the Hippo pathway to further discover suitable targets in the treatment of metastatic malignancies.

Methods: Hsp27 gain and loss of function experiments will be done on three different cancer cell lines and the functional effects on every step of the pathway will be monitored via Western blots and Immunofluorescence. Activity of YAP/TAZ after Hsp27 gain and loss of function will be monitored by conducting qRT-PCR on TEAD target genes. Transcriptional activity of TEAD1 will be examined using a TEAD-dependent Luciferase reporter construct. Stable Hsp27 knockdown PC3 cell line will be generated using short hairpin RNA (shRNA) to evaluate the long term changes in the Hippo pathway. Co-immunoprecipitation assay will be done to analyze protein interactions in the absence/presence of Hsp27. Pathway activity in prostate cancer will be assessed by immunohistochemistry staining of core components of the pathway in patients’ tissue samples.

Results: Our preliminary findings indicate that Hsp27 negatively affects the Hippo pathway. We found that targeting Hsp27 using siRNA in the prostate cancer cell line PC3 leads to increased cytoplasmic retention of p-YAP compared to control siRNA treated cells. Moreover, using Immunofluorescence, we observed reduced nuclear translocation of the YAP/TAZ as well as sequestration of these components with cytoplasmic 14-3-3 proteins in siRNA treated PC3 cells. Furthermore inhibition of Hsp287 in prostate and lung tumour cells resulted in suppression of TEAD transcriptional activity analyzed by qRT-PCR and luciferase assay.

Conclusion: Hsp27 overexpression contributes to inactivation of Hippo pathway. Inhibition of Hsp27 leads to inactivation of YAP and TAZ which results in decreased tumor cell proliferation.

Impact: Our data further supports the significance of targeting Hsp27 as a treatment option in cancers, especially metastatic malignancies like CRPC.

22. RISK OF COMPETING CAUSES OF DEATH DUE TO CHRONIC AND Cardio-Cerebro VASCULAR DISEASES AMONG ORAL CANCER PATIENTS IN BRITISH COLUMBIA FROM 1970-2009
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Objective: Oral cavity cancer (OCC) patients share common risk factors, like smoking and alcohol use, with patients with other chronic diseases while oropharyngeal cancers (OPC) can develop in patients with head and neck squamous cell carcinoma (HNSCC). Hsp27 regulates activity of several oncogenic pathways and is a molecular chaperone highly and uniformly expressed in treatment resistant cancers like castrate resistant prostate cancer (CRPC). Hsp27 regulates activity of several oncogenic pathways its levels correlate with aggressive tumor behaviour, drug resistance and tumor growth. Similarly, dysregulation of the Hippo tumor suppressor pathway, which restricts organ size and cell proliferation, occurs in many types of cancers. In healthy cells, activation of the Hippo pathway results in phosphorylation and cytoplasmic retention of two transcriptional co-activators YAP and TAZ, whereas in cancer YAP and TAZ are free to translocate to the nucleus and increase cell proliferation by promoting the activities of certain transcriptional factors including TEAD1. Inactivation of the Hippo pathway correlates with poor patient outcome and progression of tumors as well as an increase in migration, invasion and metastatic potential of cancer cells. Therefore it is of great importance to establish the correlation between Hsp27 and the Hippo pathway to further discover suitable targets in the treatment of metastatic malignancies.

Methods: Hsp27 gain and loss of function experiments will be done on three different cancer cell lines and the functional effects on every step of the pathway will be monitored via Western blots and Immunofluorescence. Activity of YAP/TAZ after Hsp27 gain and loss of function will be monitored by conducting qRT-PCR on TEAD target genes. Transcriptional activity of TEAD1 will be examined using a TEAD-dependent Luciferase reporter construct. Stable Hsp27 knockdown PC3 cell line will be generated using short hairpin RNA (shRNA) to evaluate the long term changes in the Hippo pathway. Co-immunoprecipitation assay will be done to analyze protein interactions in the absence/presence of Hsp27. Pathway activity in prostate cancer will be assessed by immunohistochemistry staining of core components of the pathway in patients’ tissue samples.

Results: Our preliminary findings indicate that Hsp27 negatively affects the Hippo pathway. We found that targeting Hsp27 using siRNA in the prostate cancer cell line PC3 leads to increased cytoplasmic retention of p-YAP compared to control siRNA treated cells. Moreover, using Immunofluorescence, we observed reduced nuclear translocation of the YAP/TAZ as well as sequestration of these components with cytoplasmic 14-3-3 proteins in siRNA treated PC3 cells. Furthermore inhibition of Hsp27 in prostate and lung tumour cells resulted in suppression of TEAD transcriptional activity analyzed by qRT-PCR and luciferase assay.

Conclusion: Hsp27 overexpression contributes to inactivation of Hippo pathway. Inhibition of Hsp27 leads to inactivation of YAP and TAZ which results in decreased tumor cell proliferation.

Impact: Our data further supports the significance of targeting Hsp27 as a treatment option in cancers, especially metastatic malignancies like CRPC.
23. DECODING THE T CELL RESPONSE TO TUMOR NEO-ANTIGENS USING LARGE SCALE RNA-SEQ DATASETS

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Somatic missense mutations can initiate tumorgenesis and, conversely, anti-tumor cytotoxic T lymphocyte (CTL) responses. Tumor genome analysis has revealed extreme heterogeneity among tumor missense mutation profiles, but their relevance to tumor immunology and patient outcomes has awaited comprehensive evaluation. Here, for 1842 patients from ten tumor sites, we performed autologous HLA prediction on RNA-seq data from The Cancer Genome Atlas (TCGA) using HLAminer1. Using these predictions, we were able to compute patient-specific mutational epitope profiles using IEDB’s MHC-I epitope binding prediction tool2. Using epitope binding and gene expression data, we identified somatic tumor mutations that were likely immunogenic (mutational epitopes presented by each patient’s MHC proteins encoded by each patient’s autologous HLA alleles). Tumors with predicted immunogenic mutations had higher CTL content, inferred from CD8A gene expression, and elevated expression of the CTL exhaustion markers PDCD1 and CTLA4.3 Mutational epitopes were very scarce in tumors without evidence of CTL infiltration.

To interrogate the clonality of the CTL response, we extracted T cell receptor (TCR) alpha and beta chains from the TCGA RNA-seq data using MiTCR4. As MiTCR was designed to analyze TCR-seq data, a stringent filtering procedure was developed to remove false-positives. This work is ongoing, but preliminary analysis of 1108 TCGA subjects revealed between 0 and 145 unique, productive, TCR sequences per patient, and the coverage of each unique TCR ranged from 1 to 44 reads. We are currently investigating the relationship between the diversity of a patient’s T cell repertoire and their total and immunogenic mutational load, HLA type, HLA expression values, and various clinical correlates, as well as investigating shared TCR sequences between patients.

References:

We thank NIH and the Cancer Genome Atlas Research Network for data access (phs000178.v8.p7). This work was supported by the British Columbia Cancer Foundation and grants from CIHR (MOP-102679) and the U.S. Department of Defense (W81XWH-12-1-0604).

24. A CRITICAL ROLE OF CAIX IN TUMOR INVASION AND METASTASIS THROUGH INTERACTION WITH AND ACTIVATION OF MMP-14 IN INVADOPODIA

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Tumor hypoxia promotes tumor cell invasion and metastasis. However, the molecular basis of hypoxia-induced invasion is poorly understood. Here we demonstrate that Carbonic Anhydrase IX (CAIX), a hypoxia induced cell surface protein involved in pH regulation is required for tumor cell invasion and formation of mature invadopodia. CAIX co-localizes with the membrane bound matrix metalloprotease, MMP14, within contactin and Tks5 positive invadopodia. The cytoplasmic domain of CAIX is required for interaction with MMP-14, and for stimulation of MMP-14-mediated matrix degradation, invasion and metastasis. Depletion of CAIX or inhibition of its catalytic activity in CAIX-expressing cells results in decreased invadopodia formation, decreased MMP-14 activity, as well as inhibition of invasion and metastasis. We have identified CAIX as a novel, hypoxia induced component of invadopodia and we demonstrate that CAIX-mediated acidification of the extracellular face of invadopodia activates MMP-14, matrix degradation, invasion and metastasis.

This work was supported by the Canadian Institutes of Health Research. Project Title: Targeting carbonic anhydrase IX and hypoxia for the diagnosis and treatment of aggressive breast cancers (Project Leader: Shoukat Dedhar).
25. REAL-TIME IN VIVO ORAL PATHOLOGY USING OPTICAL COHERENCE TOMOGRAPHY

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Objectives: Optical coherence tomography (OCT) is a non-invasive imaging technique that can generate detailed 2- and 3-dimensional in vivo images of tissue morphology. Clinical usefulness of OCT in the oral cavity has been limited because of the small (~mm) field-of-view (FOV) of previously demonstrated systems. The objectives of this study are to 1) to construct an OCT system with a large FOV probe specifically designed for oral cavity, and 2) to examine the feasibility of the instrument in capturing high-resolution images in clinical settings.

Design: Feasibility study of a newly developed OCT system in patients recruited for the COOLS Trial, a pan-Canadian Phase III randomized controlled study.

Methods: An OCT system based on rotary-pullback probes was built to capture 3D tissue OCT scans with single acquisition FOV of approximately 2.5mm wide and 40mm long in seconds. Two hand-held probe holders based on saliva ejectors and disposable dental mirrors were designed to facilitate probe placement on the lesions in the oral cavity. Tumour and margin tissue OCT images were acquired in vivo before excision, and also ex vivo on the excised tissue in the operating room. The formalin-fixed, paraffin embedded, sectioned and H&E stained specimens were used for OCT image comparison.

Result: Two patients undergoing surgery for tongue cancer were imaged with the OCT system. The surgeon noted minimal disruption to the surgical procedure due to the short OCT acquisition time (2-3 minutes) without the need to change the light setting in the operating room. Comparison of the OCT images with histology showed correlation between observed morphological features such as changes in epithelial thickness and the presence of the basement membrane. Tumour areas and high-grade dysplasias were readily distinguished from adjacent normal tissue.

Conclusions: This hand-held wide FOV OCT imaging system shows promise as a real-time pathology tool for clinicians 1) to help ensure accurate surgical removal of tumour and high-risk tissue and 2) to aid in the decision of biopsy site, especially for larger oral lesions.


26. INTEGRIN-LINKED KINASE LINKS DYNACTIN-1/DYNACTIN-2 WITH CORTICAL INTEGRIN RECEPTORS TO ORIENT THE MITOTIC SPINDLE RELATIVE TO THE SUBSTRATUM

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Cells must divide strictly along a plane to form an epithelial layer parallel to the basal lamina. The axis of cell division is primarily governed by the orientation of the mitotic spindle and spindle misorientation pathways have been implicated in cancer initiation. While 1-Integrin and the Dynactin/Dynactin complex are known to be involved, the pathways linking these complexes in positioning the mitotic spindle relative to the basal cortex and extracellular matrix remain to be elucidated. Here, we show that Integrin-Linked Kinase (ILK) and -Parvin regulate mitotic spindle orientation by linking Dynactin-1 and Dynactin-2 subunits of the Dynactin/Dynactin complex to Integrin receptors at the basal cortex of mitotic cells. ILK and the IPP complex member -Parvin are both required for proper spindle orientation in HeLa cells. ILK interacts with Dynactin-1 and Dynactin-2 and knock-down of ILK attenuates Dynactin-2 localization to the basal cell cortex. Furthermore we show that Dynactin-2 can no longer colocalize or interact with Integrin receptors when ILK is absent which suggests mechanistically that ILK is acting as a linking protein. Finally we demonstrate that mitotic spindle orientation and cell proliferation are disrupted in intestinal epithelial cells in vivo using tissue-specific ILK knockout mice. These data demonstrate that ILK, in complex with -Parvin, acts as a linker between Integrin receptors and the Dynactin complex to regulate mitotic spindle orientation.

This work was supported by the Canadian Institutes of Health Research.
27. POLYCOMB-MEDIATED SILENCING IN NEUROENDOCRINE PROSTATE CANCER
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Background: Neuroendocrine prostate cancer (NEPC) represents a highly aggressive malignancy for which the median survival is less than a year, owing to the lack of suitable pre-clinical models to study its molecular basis. Addressing this need, we have previously established a high-fidelity, patient-derived xenograft model of NEPC (331R) arising from the transdifferentiation of a prostate adenocarcinoma (331) following androgen-deprivation therapy in vivo. Our initial analysis revealed that 331 and 331R shared a remarkably similar genetic profile, suggesting that epigenetic alterations were likely to drive NEPC pathogenesis.

Objectives: The underlying goals of this research project were to characterize the epigenetic landscape of NEPC and to identify potential epigenetic drug targets for this lethal disease.

Methods: We conducted comparative gene expression analysis between 331R and 331, as well as a clinical cohort, to identify epigenetic regulators that were differentially expressed in NEPC. We also investigated genes that were consistently downregulated in many NEPC models to infer their molecular and biological properties.

Results: By combining expression profiles from multiple NEPC datasets, we provided evidence that multiple PcG family members are overexpressed in NEPC, notably CBX2 and EZH2. Furthermore, CBX2 and EZH2 were specifically upregulated in small cell lung cancer, a neuroendocrine tumor used as a model for NEPC, compared to non-small cell lung cancer. Consistent with these results, we derived a neuroendocrine-associated repression signature (NEARS) that was enriched in PcG targets and significantly predicted aggressive progression in clinical prostate cancer cohorts.

Conclusions: Overall, we have explored the epigenetic landscape of NEPC in unique models derived from patient tissues. Our results support a clinically-relevant function for PcG-mediated silencing in NEPC, providing novel avenues for epigenetic therapies in the context of lethal NEPC.

28. SMALL CELL CARCINOMA OF THE OVARY, HYPERCALCERCIAL TYPE DISPLAYS FREQUENT INACTIVATING GERMLINE AND SOMATIC MUTATIONS IN SMARCA4
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Introduction: Small cell carcinoma of the ovary, hypercalcerical type (SCC(O)HT) is arguably the most aggressive ovarian cancer. Most patients are diagnosed at an advanced stage, do not respond to chemotherapy, and die of disease within 1-2 years. It affects children and young women and is reported to occur in families. The cause of the disease is poorly understood. Therefore, we used next generation sequencing technology to identify the genetic basis of the disease.

Methods: Tumour and germline DNA samples from 17 SCC(O)HT patients and on 2 SCC(O)HT cell lines (BIN-67 and SCC(O)HT-T1) were analysed by whole genome, whole exome sequencing or targeted sequencing. Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded tumours from 23 patients and on a tissue microarray of 485 primary ovarian tumours of other subtypes. BIN-67 cells harbouring biacryl inactivation of SMARCA4 were transduced with a lentivirus expressing wild type SMARCA4.

Results: We identified inactivating germline and somatic mutations in the SWI/SNF chromatin-remodeling gene SMARCA4 in 79% (11/14) of SCC(O)HT patients, 2 of whom bore germline mutations, and in both cell lines. SMARCA4 protein was lost in 87% (20/23) of SCC(O)HT tumours but in only 0.4% (2/485) of other ovarian tumours. Reintroduction of wild-type SMARCA4 into BIN-67 cells resulted in altered cell morphology and growth arrest.

Conclusions: The mutation spectrum, IHC profile and cell culture phenotype implicate SMARCA4 as a critical tumour suppressor in SCC(O)HT pathogenesis. This work is supported by the Terry Fox Research Institute’s TFF New Frontiers Program in Cancer grant. Project title: CIHR Team in Genomics of Forme Fruste Tumours: New Vistas on Cancer Biology and Treatment.
29. HIGH-THROUGHPUT DRUG SCREEN IDENTIFIES HDAC, PI3K AND PROTEASOME COMPLEXES AS POTENTIAL TARGETS IN THE TREATMENT OF SYNOVIAL SARCOMA


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Introduction: Conventional cytotoxic therapies for synovial sarcoma provide limited benefit and no drugs specifically targeting the driving SS18-SSX mutation are currently available. Patients remain at high risk for early and late metastasis, leading to a mortality rate of approximately 50% within 10 years of diagnosis. SS18-SSX represses PTEN and synovial sarcomas express high levels of BCL2, leading to an apoptosis-resistant phenotype. HDAC inhibitors are known to restore PTEN as well as induce degradation of SS18-SSX, though trials in patients with advanced disease have not shown clinical responses. A high-throughput drug screen was undertaken in a panel of synovial sarcoma cell lines to uncover novel sensitizing agents and targetable pathways.

Methods: A 900 compound library comprised of OICR tool compounds, tyrosine kinase inhibitors and epigenetic modulators was screened on panel of fusion oncogene-confirmed cell line models: six synovial sarcoma, three myxoid liposarcoma, two clear cell sarcoma and one Ewing sarcoma; as well as one breast cancer and one normal cell control, utilizing a 96-well multi-blotter technique. At 48 hours post treatment, screens were developed with MTS reagent and cell viability was assessed by spectrometry. Compound efficacy was evaluated based on potency and specificity in all synovial sarcoma cell lines. Validation studies were undertaken for the top scored compounds: Annexin-V-PI and cleaved caspase 3/7 apoptosis assays, cell imaging viability assays and spheroid treatment studies. Changes in expression of key synovial sarcoma genes were assessed at the mRNA and protein level. Combinational studies were utilized to measure potential compound synergy.

Results: Among the 100+ surveyed drug classes, HDAC inhibitors, PI3K/AKT/mTOR inhibitors and proteasome targeting agents were the most effective against synovial sarcoma. Top hits were validated on a panel of synovial sarcoma cell lines and were shown to be more potent than the currently used cytotoxic agents. Functional data demonstrated that the HDAC inhibitor quisinostat blocks key SS18-SSX mediated effects, including EGR1 and PTEN repression as well as BCL2 over-expression. By comparison, other drug categories were less effective in synovial sarcoma cell lines, including Wnt and MDM2 inhibitors.

Conclusion: In a high-throughput drug screen of 900 compounds, top scored agents in decreasing synovial sarcoma cell viability were found to be HDAC inhibitors, PI3K inhibitors and proteasome targeting agents.

30. CHARACTERIZING ERG-MEDIATED TRANSCRIPTIONAL REPROGRAMMING OF PROSTATIC EPITHELIAL CELLS TOWARDS A CANCER STEM CELL PHENOTYPE

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ERG expression commonly occurs in prostate cancer (PCa). While often linked to poor prognosis, how ERG might mechanistically promote transformation remains to be elucidated. ERG-expressing RWPE1 prostatic epithelial cells (ERG-RWPE1) were constructed to test whether ERG transformation is caused by specific transcriptional profile changes. ERG induces RWPE1 transformation and ILK-mediated epithelial-to-mesenchymal transition. Microarray analysis of ERG-RWPE1 cells demonstrated up-regulation transcription of stem cell (SC)-related genes. Expression of the SC markers PROM1, c-KIT and CD33, were validated, but was limited to a minority of the adherent population. ERG-RWPE1 cells form self-renewable spheroids when cultured in non-adherent SC media. Spheroids uniformly express PROM1, c-KIT and CD33 and when replated in adherent conditions, reestablish the SC marker expression pattern of the parental adherent cultures. Transcript analysis of ERG-RWPE1 spheroid cultures also revealed activation of transcriptional reprogramming events related to acquisition of SC-like capacity, including up regulated expression of a panel of “Yamanaka-related” SC genes (the sentinel genes: OCT3/4, SOX2, MYC and KLF4), epigenetic control (PRC1, PRC2 and Homeobox C family genes) and genes belonging to DNA methyltransferase, long noncoding RNA and hypoxia pathways. Our results demonstrating that ERG induces a transcriptional reprogramming resulting in acquisition of SC characteristics in RWPE1 cells, establish this as a model to interrogate molecular mechanisms of transformation and design of new therapies in prostate cancer.

This work is supported by the Terry Fox Research Institute/Canadian Institutes of Health Research Program on Prostate Cancer Progression (PI: Martin E. Gleave).

Becker-Santos et al., Carcinogenesis 2012.
31. RESOURCE UTILIZATION AND COSTS DURING THE INITIAL YEARS OF LUNG CANCER SCREENING WITH COMPUTED TOMOGRAPHY IN CANADA


Resource utilization data were collected prospectively from 2,059 participants in the Pan-Canadian Early Detection of Lung Cancer Study using low-dose computed tomography. Participants who had ≥2% lung cancer risk over three years using a risk prediction tool were recruited from seven major cities across Canada. A cost analysis was conducted from the Canadian public payer's perspective for resources that were used for the screening and treatment of lung cancer in the initial years of the study.

Results: The average per-person cost for screening individuals with low dose computed tomography was $453 (95% CI: $43,254-$52,200) (p=0.061). It is estimated that millions of North Americans would qualify for lung cancer screening and that billions of dollars of national health expenditures would be required to support population-based computed tomography lung cancer screening programs. The decision to implement such programs should be informed by data on resource utilization and costs.

Acknowledgement: This project was funded in part by the Terry Fox Research Institute, the Canadian Partnership Against Cancer, with co-funding by the Princess Margaret Cancer Foundation Louis Wong Fund (Toronto site). The Canadian Centre for Applied Research in Cancer Control (ARCC) is funded by the Canadian Cancer Society Research Institute.

32. TOWARDS THE OPTIMIZATION OF NON-INVASIVE AND COMPREHENSIVE METHODS TO QUANTIFY AND CHARACTERIZE CIRCULATING TUMOUR DNA

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Ultrasensitive methods for detecting mutant DNA molecules in the plasma, including those relying on deep sequencing, have recently revealed the potential of circulating tumour DNA (ctDNA) as a biomarker for solid cancers. Sequencing-based methods offer potential for the non-invasive characterization of mutations associated with disease that occur prior to diagnosis and may further allow detection of genetic alterations or clonal expansions that occur during treatment.

Common limitations of sequencing-based ctDNA detection include low detection thresholds hampered by sequencing and/or biological background noise, non-optimal discrimination among unique DNA molecules and the difficulty to efficiently detect genetic rearrangements. We present improvements to sequencing-based approaches for ctDNA detection that enable precise quantification of mutant DNA molecules while aiming to suppress artifacts introduced during PCR and sequencing. Capture and single-molecule tagging of plasma-derived cell-free DNA, using starting amounts as low as 5 ng, is accomplished through overnight ligation in the presence of a 100-fold molar excess of custom barcoded adapters. In cancer types characterized by single nucleotide variants (SNVs), libraries are enriched for the exons of genes known to be recurrently mutated using a pool of custom barcoded DNA probes. For cancers such as sarcomas, which are characterised by specific semi-recurrent chromosomal rearrangements, we produce populations of biotinylated RNA baits that span intronic regions that typically harbour rearrangement breakpoints. Using such assays, we have studied the level of ctDNA in diffuse large B-cell lymphoma (DLBCL) patients using individual reporter somatic mutations such as SNVs and indels. We have also captured the genomic rearrangement underlying gene fusions in synovial sarcoma diluted in wild type DNA to mimic ctDNA. Preliminary analyses indicate that our library preparation method captures up to ~41% of the DNA fragments present in a plasma sample. Our capture protocol allows up to 45,000-fold enrichment for targeted genes allowing mutant allele fractions ranging from 0.25 to 26% to be detected in plasma. Such analyses relied on the design of specialized workflows that can be launched from the user-friendly Geneious (Biomatters Ltd) genomic workbench. These improvements in the detection and quantification of ctDNA should strengthen our capabilities to non-invasively assess tumour burden and hold promise to detect ongoing clonal evolution in tumours in response to treatment.


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33. LYN KINASE STABILIZES SNAI FAMILY TRANSCRIPTION FACTORS AND INDUCES EMT

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Introduction: Metastasis is the most common cause of death from cancer and occurs when malignant cells discard epithelial restraints and acquire invasive abilities, facilitating their dissemination to permissive micro-environments. This process is enhanced by tumor cell activation of Epithelial Mesenchymal Transition (EMT), a normally embryonic developmental program in which epithelial cells assume a mesenchymal phenotype during gastrulation and organogenesis, allowing single cell invasive movement away from the ectodermal layer. Recent evidence strongly implicates EMT induction in malignant progression and treatment resistance. For example, EMT regulatory transcription factors are required for breast cancer metastasis. Several oncogenic pathways (growth factors, Src family, MAPK, AKT) induce EMT. Lyn tyrosine kinase, a member of Src family tyrosine kinase is up-regulated in advanced prostate cancer and has been reported to correlate with aggressive breast cancer. Our objective is to determine the role of Lyn tyrosine kinase in EMT.

Methods: LNCaP (Prostate Cancer), BT-549 (Breast Cancer), UC-13 (Bladder Cancer) cells were transfected with Lyn siRNA. EMT markers were monitored by western blot and RT-PCR and immunofluorescence. Migration by scratch assay, invasion by Boyden chamber and matrix metalloproteinase (MMP) activity by Zymography. Lyn specific Kinase inhibitor Bafetinib will also be used along with over-expression experiments with Lyn Kinase Dead and Constitutively active mutants. 3D cell culture in matrigel is also used to measure invasion potential.

Results: Here we report that Lyn expression is low in epithelial cells and is up-regulated in mesenchymal cells. Targeting Lyn using siRNA decreases EMT markers (Fibronectin, Vimentin) at both mRNA and protein levels while increasing the epithelial marker (E-cadherin). Moreover, we found that targeting Lyn by siRNA/miRNA and small molecule inhibitor Bafetinib decreases cell migration, invasion and the activity of MMPs. This decrease in mesenchymal phenotype can be attributed to the decrease in the amount of Slug and Snail, transcriptional repressors of E-Cadherin and activators of Vimentin. Consequently, we also found that over-expressing Lyn in both as WT or constitutively active (CA) mutant rescues expression of Slug and Snail, inducing EMT, increased cell migration and invasion while Kinase Dead (KD) mutant has no effect.

Conclusion: Expression of Lyn kinase can be correlated to low prognosis and aggressive/metastatic phenotype. We show that targeting Lyn activity initiates a switch to a more epithelial phenotype reducing cell migration and invasion.

Impact: The data suggests that Lyn tyrosine kinase plays a role in Epithelial Mesenchymal Transition and could be considered as a target for metastatic disease; especially in the more aggressive forms of cancer like Triple Negative Breast Cancer or Castration Resistant Prostate Cancer. This could be a realistic therapeutic option, as the Lyn small molecule inhibitor Bafetinib, is currently in clinical trials for treatment of several cancers.

This work was supported by the Terry Fox Research Institute.

34. MECHANISMS AND IMPACTS OF EXOSOMAL MICORNAS ON LUNG ADENOCARCINOMA TUMORIGENESIS

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Background: Lung cancer is the leading cause of cancer death worldwide. With a low five-year survival rate it is important that new methods for treatment are discovered. microRNAs (miRNA) are small non-coding RNA molecules that modulate the activity of specific protein coding genes. miRNAs have been shown to have a number of functions in cancer cells including playing a tumor suppressive and an oncogenic function. miRNAs are packaged into exosomes non-randomly as certain miRNAs are highly enriched in exosomal fractions compared to cellular fractions. Exosomes have been shown to have considerable function in the progression of many cancers including lung cancer.

Objective: To discover the impact of exosomal miRNAs on lung adenocarcinoma tumorigenesis.

Methods: miRNA profiles were generated from exosomal and cellular fractions of lung adenocarcinoma cell lines. Candidate miRNAs that have at least a four-fold change between the two fractions were selected for further analysis. To assess the biological role of the miRNA candidates selectively packaged into exosomes, lentiviral miRNA inhibitors were added into the lung adenocarcinoma cell lines to knockdown candidate miRNAs. After selection for knockdown GW4869, a drug that inhibits exosome formation was added into the cell lines in order to prevent exosomes formation, trapping exosomal miRNAs within the cells thereby increasing their concentration and allowing us to determine miRNA intracellular function.

Results: Seven miRNAs are up regulated in exosomal samples while only one was up regulated in cellular fractions. miRNAs were successfully knocked down by lentiviral inhibitors with ~90% efficiency allowing us to assess function and role in cell growth. miRNAs up-regulated in exosomes appear to regulate key oncoprogens, including EGFR and c-Myc.

Conclusion: We have identified a novel mechanism of gene regulation through miRNAs actively being selected for export via exosomes. These miRNAs function to regulate key oncogenes within the tumor and it is predicted that exosomal packaging of miRNAs eliminates tumor suppressive miRNAs from the cell. This is a novel form of gene regulation that could be exploited for the development of novel therapeutics.

This research is funded by the Terry Fox Research Institute. Project title: Circulating microRNAs as a lung tumour proxy: determining whether a small RNA species in plasma can be used as an early cancer detection tool.
We report opposing effects of CD163+ macrophages on outcome. This discrepancy could be explained...
37. TARGETING CHOLESTEROL IN ADVANCED PROSTATE CANCER (PCA) IS CORRELATED WITH REDUCED PSA AND ANDROGEN LEVELS, DECREASED GROWTH AND DECREASED PROGRESSION IN CELL AND MURINE MODELS

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Introduction: Existing therapies for advanced metastatic PCA provide clinical benefit; however, the achieved remission is usually temporary with recurrence and progression to the lethal and treatment resistant castration-resistant PCA (CRPC). Cholesterol is thought to play key roles in CRPC as an essential factor for membrane structure, organelle function and cell signaling and as the precursor for de novo steroidalogenesis. PCA progression is associated with elevated expression of the rate limiting step in cholesterol biosynthesis, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), and the high density lipoprotein receptor, scavenger receptor B-I (SR-BI); both are upregulated in LNCaP tumors isolated from LNCaP xenograft mice as well as in the CRPC C4-2 cell line, relative to androgen sensitive LNCaP cells. Herein, we describe studies using human PCA cell lines as well as the LNCaP xenograft mouse model to assess the effects of cholesterol limitation in CRPC growth and progression. Cholesterol blockade was achieved by targeting SR-BI in vitro and by inhibiting cholesterol synthesis with simvastatin in vivo.

Methods: LNCaP and C4-2 cells were maintained in RPMI-1640 medium supplemented with 10% FBS. SR-BI knockdown experiments were performed in C4-2 cells cultured in RPMI-1640 medium supplemented with either 1% FBS or 5% charcoal stripped serum. C4-2 cells were transfected using Lipofectamine® with either stealth RNAi duplexes targeting the SR-BI gene or negative control duplexes. Cells were grown for 6 days post-transfection; media samples were collected for prostate specific antigen (PSA), chemiluminescence, cell lysates were collected for protein (western blot), cholesterol (fluorometry), and sterol ILC-MS/MS analyses. DNA staining with propidium iodide was used to assess cell cycle arrest and apoptosis. For in vivo work, Nu/nu mice were inoculated with LNCaP cells and allowed to develop tumors. Upon reaching a PSA of at least 50 ng/ml, the animals were castrated and started on either a 0.1% w/v simvastatin chow or a standard diet. Tumor volume was assessed through caliper measurements once weekly. Circulating PSA and intratumoral steroids were measured as above. Potential simvastatin toxicity was assessed by alanine transaminase and creatine kinase assays.

Results: Over 80% knockdown of SR-BI protein was achieved which was correlated with decreased PSA and proliferative arrest of C4-2 cells. Total cellular cholesterol levels did not change but testosterone and dihydrotestosterone concentrations were decreased in the knockdown group. Mice placed on the simvastatin chow showed reduced tumor growth, with the greatest differences being displayed during weeks 6-8 post-castration. The simvastatin group also had a longer time to 400% of nadir PSA with almost half of the standard group reaching this level within 3 weeks post castration compared to 10% of the simvastatin group. Decreased tumor growth was correlated with decreased intratumoral testosterone and dihydrotestosterone levels.

Conclusion: Restriction of cholesterol suppresses growth of LNCaP derived models under androgen-deprived conditions both in vitro and in vivo. Due to its roles as an essential factor in cell biogenesis and as a precursor for intratumoral androgen synthesis, we conclude that targeting cholesterol may have benefit by complimenting current maximal androgen blockade strategies.

This work was supported with funding from Prostate Cancer Canada.

38. ANALYSIS OF RELAPSE BIOPSIES IN CLASSICAL HODGKIN LYMPHOMA REVEALS CORRELATIONS WITH OUTCOME AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction: Classical Hodgkin lymphoma (CHL) is the most common lymphoma affecting individuals under the age of 30. Despite improvements in standard treatment, 25-30% of patients still relapse after first-line treatment with ABVD. Relapsed patients are then typically treated by high dose chemotherapy and autologous stem cell transplantation (HDC/aSCT). However, this salvage therapy only cures approximately 50% of relapsed patients, and it is unclear what the mechanisms of second-line treatment failure are. Moreover, currently no reliable biological markers are available to predict the outcome of aSCT at the time of relapse. The specific aim of this study is to compare diagnostic and relapse biopsies along with integrating clinical outcome data to uncover biology associated with resistance to first-line therapy, and identify prognostic markers of aSCT.

Materials & Methods: Nanostring digital gene expression profiling was used to ascertain the gene expression of 785 patients with CHL. Comparisons were made between the relapse and diagnostic biopsies of the same patient. The relapse and diagnostic biopsies were compared to the relapse biopsies. Using the published thresholds, 19% of patients were designated high-risk and had significantly inferior post-aSCT survival (P = 0.005). Additionally, we found to be more highly expressed in the relapse biopsies as compared to the matching samples at first diagnosis. Using this information, we developed a newly constructed 23-gene predictor which was applied to the cohort of over 200 patients which was validated on an independent set of 80 patients.

Results: 24% of patients had a histological subtype transition between diagnostic and relapse biopsies. Of the patients with subtype transition, the most common transition (46%) was from mixed cellularity (I) to nodular sclerosis (II). Comparative gene expression analysis revealed that 17 of the 71 patients (24%) had poorly correlated biopsy pairs (R2 < 0.75). Specifically, genes associated with macrophage differentiation (e.g. CD68, MARCO; FOR < 0.1) were found to be more highly expressed in the relapse biopsies as compared to the matching samples at first diagnosis. Patients with poorly correlated biopsy pairs had an inferior post-aSCT failure-free survival (P = 0.005). Additionally, we applied our previously published 23-gene predictor (Scott et al., JCO. 2013), that was originally developed for diagnostic biopsies, to the relapse biopsies. Using the published thresholds, 19% of patients were designated high-risk and had significantly inferior outcomes post-aSCT (P = 0.001).

Conclusions: Our comparative analysis of chl diagnostic and relapse biopsies reveals differences at both the histopathological and molecular level. We have identified novel factors, such as a poor correlation between paired biopsies and the 23-gene predictor, to be predictive of an inferior post-aSCT outcome. These findings suggest that re-biopsying patients at relapse will yield important information and thus superior predictive power for second-line treatment failure.
39. THE ROLE OF CCR5+ REGULATORY T CELLS IN BREAST CANCER METASTASIS

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Background: In 2014, it is estimated that 24,400 Canadian females will be diagnosed with breast cancer, and approximately 5,000 of these women will succumb to their disease. The development of distant metastases is largely responsible for the mortality associated with breast cancer. Regulatory T cells (Tregs) are an immune suppressive population associated with the growth and metastasis of mammary tumours. Under homeostatic conditions, CD4+CD25+Foxp3+ Tregs function to mediate peripheral tolerance and prevent autoimmunity; however, elevated proportions of tumour-infiltrating Tregs are associated with poor prognosis in several solid tumour types. A potential therapeutic strategy to decrease primary or metastatic tumour growth may be to target Tregs, although therapies that systematically deplete Tregs have been linked to the development of autoimmunity. Therefore, research to specifically inhibit the homing or suppressive function of tumour-infiltrating Tregs is warranted to minimize the off-target effects of systemic Treg depletion. Our preliminary data suggests that C-C chemokine receptor type 5 (CCR5) is highly and selectively expressed by tumour-infiltrating Tregs. The goal of this research is to evaluate the contribution of CCR5 expressing Tregs to breast tumour metastasis and the development of pulmonary metastatic foci. By studying the role of CCL8 and IL-16, two factors known to recruit CCR5+ cells, in recruiting CCR5+ Tregs to metastatic target organs, we hope to design novel therapeutic strategies for metastatic cancer.

Hypothesis: We hypothesize that CCL8 and IL-16 promote the migration of immune suppressive CCR5+ Tregs to the primary tumour and metastatic tissues, and that the inhibition of CCR5+ Tregs will decrease primary tumour cell metastasis and metastatic tumour growth.

Aims: 1) To examine the mechanism of recruitment and immune suppressive function of CCR5+ Tregs in the primary tumour and metastatic target organs. 2) To inhibit CCR5, IL-16 or CCL8 in vitro and in vivo and observe the effect on Treg recruitment, tumour cell metastasis, and the development of metastases.

Results: We found that the lungs and tumours of mice bearing metastatic breast tumours accumulate significantly more CD4+CD25+Foxp3+ Tregs than naive, non tumour-bearing mice. The proportion of Tregs was found to increase with time post tumour cell implantation. Analysis of chemokine profiles indicated that the production of CCL8 and IL-16 were increased in the primary tumour and lung tissue relative to naive mice. CCL8 levels were quantified and found to be elevated in the bronchoalveolar lavage, supernatant and lysate from the lungs of mice bearing metastatic breast tumours relative to naive control lungs. Correspondingly, we found that CD4+CD25+Foxp3+ Tregs in the tumour and metastatic lungs expressed high levels of CCR5. Chemotaxis assays demonstrated that ex vivo isolated Tregs migrate toward a gradient of CCL8 and IL-16 in vitro and this migration could be inhibited by treatment of Tregs with the CCR5-specific inhibitor Maraviroc. Subsequent experiments are underway to examine the immune suppressive activity of CCR5+ Tregs, to test the efficacy of Maraviroc as an inhibitor of metastasis in vivo, and to inhibit CCR5, IL-16 or CCL8 in vitro and in vivo.

Significance: This project seeks to advance the development of targeted, immune-based therapeutics for the treatment of metastatic breast cancer. Ultimately, we hope to provide important information about the role of CCR5+ Tregs in metastasis to develop novel therapeutic strategies to treat metastatic cancer.

This work is supported by the Terry Fox Research Institute. Project title: Promotion of metastasis by bone marrow-derived cells.

40. DIFFERENT GROWTH PROPERTIES OF EPITHELIAL PROGENITOR CELLS ISOLATED FROM THE CRYPT AND SURFACE EPITHELIUM OF NORMAL HUMAN TONSILS

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Cancer of the oropharynx (including the tonsils and base of the tongue) is a global health problem with an increasing incidence and prevalence worldwide especially in developed countries. Human Papillomavirus DNA is present in up to 90% of oropharyngeal cancers, and initial HPV integration has been localized to the epithelium of tonsillar crypts. This suggests that a primitive epithelial cell in this region may be the cell of origin of HPV-associated oral cancers. Numerous studies have demonstrated the presence of stem cells in other human epithelia but those responsible for sustaining the tonsillar crypts have not been identified.

We first used in situ immunohistochemical analysis to identify differentially expressed markers on surface of tonsillar epithelial cells and now show how these can be used to obtain highly purified suspensions of both surface and crypt epithelial cells with in vitro clonogenic ability (CFCs). The results show that prospectively isolated CD45-CD31-CD44+NGFR+ cells from either surface or crypt epithelial tissue, when cultured in 5% O2 at low cell numbers in the presence of irradiated NIH3T3 cell feeders in medium containing a ROCK inhibitor generate colonies at an average frequency 14 % for surface CFCs and 21 % for crypt CFCs (estimated from n=4). These cells were also able to form multilayered epithelial sheets when seeded onto fibroblast embedded collagen matrix in 3D organotypic cultures. In order to examine the self-renewal ability of these progenitors, we performed serial re-plating experiments; the results revealed that progenitors isolated from crypt contained higher proportion activity that generated colony type dubbed holoclones.

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Our study sets the stage for investigating intrinsic properties regulating this clonogenic subset of tonsillar epithelial cells, and their response to HPV infection, as a first step toward elucidating the pathogenesis of HPV-associated oral cancers.
41. ACTIVATING IL4R MUTATIONS IN PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA

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Introduction: Primary mediastinal large B cell lymphoma (PMBCL) is a distinct subtype of aggressive B cell lymphoma that arises from thymic medullary B cells and characteristically presents as a mass in the anterior mediastinum. A proportion of PMBCL patients are suffering from refractory or relapsing disease, and subsequent salvage therapies are frequently not effective. Development of targeted therapeutic approaches is impeded by the lack of knowledge about the mutational landscape in the lymphoma genomes and mutation-associated phenotypes. We recently reported somatic mutations in the transcriptome of 7 PMBCL patients and 3 cell lines by next-generation sequencing and found a hotspot mutation in exon 8 of IL4R (Gunawardana et al., Nature Genetics, 2014). Interleukin-4 (IL4) is a type II cytokine known for its role in the regulation of immune responses through binding to a receptor complex consisting of the IL4 receptor alpha (IL4R) chain and IL13Rα or the common gamma chain γc (depending on cellular context). Activation of IL4R by IL-4 and/or IL-13 engagement initiates intracellular signal transduction mediated by the phosphorylation of JAK/STAT Transducer and Activation of Transcription (JAK-STAT) pathway. As overactive receptor tyrosine kinases are reported critical oncogenic events in cancer, we hypothesize that constitutively active JAK-STAT observed in PMBCL are in part due to mutated IL4R.

Methods and samples: A sequencing cohort consisting of fresh-frozen lymph node biopsies from 62 PMBCL cases were selected from BCCA tissue archives. DNA from PMBCL samples and 3 PMBCL-derived cell lines were extracted for IL4R exonic PCR amplification and Sanger sequencing or high-throughput sequencing on a MiSeq instrument. Wild type and mutant (I242N) IL4R cDNA were cloned into the mammalian expression vector EF-GFP and expressed in engineered HEK-293 cells expressing STAT6. Supernatant from cultured cells were used for measuring the activity of STAT6-dependent secreted embryonic alkaline phosphatase (SEAP) and enzyme-linked immunosorbent (ELISA) assays for soluble IL4R levels. Extracted RNA from transfected cells were used for qRT-PCR and Western blotting, respectively.

Results: Somatic IL4R mutations were found in 18 of 65 (28%) cases confirming a hotspot mutation (I242N) in exon 8 in 11 of 65 (17%) cases. Ectopic expression of the mutant showed increased SEAP levels compared to WT (fold change 20 vs. 2.7 compared to empty vector control) indicating activation of STAT6 independent of cytokine stimulation. Hyperphosphorylation of STAT6 was confirmed by Western blotting on cell lysates using a monoclonal antibody against IL4R. Soluble IL4R levels determined by ELISA were significantly increased in mutant culture supernatants compared to WT (without IL4 stimulation 229% vs.100% and with IL4 stimulation 236% vs. 104%).

Discussion: IL4R mutations are recurrent in PMBCL. Functional analyses show these mutations lead to hyperphosphorylation of STAT6 and activation of the JAK-STAT pathway. Co-transfection experiments involving IL13Rα and c are currently underway to determine if the association of these partner receptors with mutant IL4R is altered and if it affects JAK-STAT signaling. These data suggest IL4R mutations as novel driver alterations in PMBCL and might provide a rational therapeutic target.

This work is supported by TFRI and CIHR.

42. TUMOUR NEST ARCHITECTURE, NOT TUMOUR THICKNESS, PREDICTS NODAL STATUS FOR EARLY-STAGED ORAL CANCEERS USING QUANTITATIVE TISSUE PATHOLOGY (QTP)

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Objectives: Oral cancer (OC) patients face a 1-in-3 risk of developing cancer to regional lymph nodes (LNs). A prophylactic neck dissection for high-risk patients may save lives. The purpose of this study is to quantitatively evaluate morphological and architectural features of tumour nests (TNs) between LN-negative and LN-positive cases using QTP.

Design: Prospectively collected, retrospective case-control study with surgical samples collected from the COOLS Trial, a Pan-Canadian multi-centre phase III randomized control study.

Methods: Tumor blocks from 15 surgical samples with known LN status were sectioned, stained with Feulgen-Thionin, and imaged. TNs were identified and demarcated on the images by a pathologist, and computer analyzed using pixel-based segmentation on the overall optical density (OD) of each nucleus. Demographic and pathological features were compared between the LN-negative and LN-positive groups using nonparametric Mann-Whitney for continuous variables and Fisher’s exact test for categorical variables. Each TN was analyzed in layers (TNLs). QTP features were first compared with nested ANOVA adjusted by patient and TN effects, followed by multivariate logistic regression and receiver operating characteristics (ROC) curve analysis. P-value < 0.05 (2-sided) was considered significant.

Results: A total of 15 patients (LN-negative, N=8; LN-positive, N=7) were included and mostly female (66.7%), non-smokers (60.0%), and with tumour site on the tongue (93.3%). There was no significant difference in clinic-pathological variables, including the commonly adapted predictor, tumour thickness (LN-negative, 9.25±7.63 mm vs. LN-positive, 6.71±3.35 mm, P=0.67). Collectively, 315 TNLs (157 LN-negative; 158 LN-positive) from 45 TNs (23 LN-negative; 22 LN-positive) were analyzed for 186 morphological and 38 architectural QTP features. Using ANOVA, 100 out of 224 features showed significant difference between LN-negative and LN-positive (P<0.05) TNLs. Among these, 10 features with area under curve (AUC) of 0.80 showed sensitivity of 0.80 and specificity of 0.80 are associated with nodal disease. When we examined layer by layer, the features (OD and texture) from the combination of the 2nd layer and 3rd outermost layers provided the best predictor for nodal status, with AUC of 0.90, sensitivity of 0.75, and specificity of 0.91.

Conclusion: QTP, with ability to automatically analyze over 200 features, has shown its potential in predicting the nodal status of OC. Further validation using these identified key features might provide a useful marker in justification of prophylactic neck dissection for early-staged OC patients and, subsequently, better survival can be reached.


4TH ANNUAL TFRI BC NODS RESEARCH DAY / ABSTRACTS
OVERCUT OR UNDERCUT - USING QUANTITATIVE TISSUE PHENOTYPE ANALYSIS TO ASSESS RISK OF RECURRENCE AT SURGICAL MARGINS

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Objectives: Quantitative Tissue Phenotype (QTP), an objective and reproducible approach to quantify nuclear phenotypic features, has been associated with degree of dysplasia and has shown its potential to predict risk of progression and recurrence in oral cancers. The objectives of this study are to use QTP to assess the risk 1) at clinically occult but fluorescence visualization loss (FVL) margins, and 2) between near (M-near) and far (M-far) surgical margins, 5 or 10 mm away from clinical and/or FV margins.

Design: Surgical margin samples were collected from the COOLS TRIAL, a Pan-Canadian multi-centre phase III randomized controlled study.

Material and Methods: Samples were collected from the anterior, posterior, medial, and lateral directions of each surgical specimen along with pathology interpretation and FV status. Some surgical margins with no clinical or FV alterations were further separate into 2 sets – M-near (a 5mm) and M-Far (between 5mm and 10 mm) beyond the tumoral margins determined by clinical and FV interpretation. Four-um sections were cut and labeled with Feulgen-thionin staining. The slides were scanned with Getafics, our in house high resolution imaging system. An automated Nuclear Phenotypic Score (aNPS), a Random-Forest based voting score, measure of changes in nuclear morphology and nuclear DNA chromatin texture was calculated for each Region Of Interest. Based on an independent training set of oral premalignant lesions with known outcome of progression to cancer, specimens associated with an aNPS higher than 0.5 were classified as high-risk. The QTP based score (aNPS) along with FV status and between M-near and M-far was examined by mixed effects models. The McNemar test was also used to compare risk levels (high risk with aNPS > 0.6) in paired margin samples. All tests were two-tailed with 0.05 as significance level.

Results: A total of 884 margins/directions with histology from 207 patients were analyzed of which 4.8% had moderate dysplasia and 95.2% had mild dysplasia/no dysplasia. Controlling for the effect of degree of dysplasia by adding it to the model as a confounding variable, although it was not found to be significant, FVL samples were more likely to have higher aNPS than those have no change in FV (0.52±0.03 vs. 0.49±0.01). Additionally, for those 198 margins available for paired M-near and M-far analysis, there was no statistically significant difference in risk as measured by aNPS (0.52±0.02 vs. 0.50±0.02; P = 0.09).

Conclusion: This study shows positive association between FVL status and aNPS risk of progression. The subtle difference in aNPS between surgical margins at 5mm and 10mm suggests that the current standard of practice of 10 mm surgical clearance could be open for discussion. The COOLS trial will gather more definitive evidence to use aNPS as a surrogate to predict recurrence as well as determine the benefit and risk of altering surgical margin delineation.


MULTI SPECTRAL IMAGING PROJECT

Gallagher P., Lam S., Poh C., and MACAULAY C.
BC Cancer Agency.

Objective: The project’s goal is to extract more information out of imaging H&E stained lung and oral samples for diagnostic purposes than is possible with other imaging techniques. We propose to accomplish this by measuring differences in H&E fluorescence depending upon which molecular components the stains are bound to. Currently it is not known how many separate molecular components can be identified in this fashion.

Methods: For this project excitation light from 410nm to 530nm in 20nm steps(7 excitation conditions) was used to illuminate the slides. A camera, with a tunable light filter, imaged the fluorescence response in the 600nm to 720nm wavelengths in 20nm steps collecting 7 images for each of the excitation wavelengths. Flat field images (uniform fluorescence target) were taken and dark field images generated. An image correction program then removes the dark field pattern noise, corrects for illumination non-uniformity using the flat field image, aligns, crops, and normalizes the illumination energy of the 49 image stack. Data for 159 locations across 48 sections has been collected. The data was then subjected to two separate data analysis techniques designed to elucidate what unique information can be extracted from these image stacks.

Matlab was used to perform Principal Component Analysis on all of the data stacks. This creates a 49 by 49 weight coefficient matrix which is applied to each stack separately. This is done to allow direct comparison between image stacks. One method of visualizing the results is to combine the first 3 principle components in a reduction in data dimensionality such that the majority of image variance can be observed in a single colour image.

The other applied technique is spectral un-mixing. Un-mixing attempts to isolate all the different substances in the sample and display them as concentration maps based on their spectra. This is done by decomposing the data into concentration and spectra matrices. With known spectra for each substance to be unmixed it is possible to calculate the corresponding concentration matrix which explains where in the image like spectra are located. However this presupposes one knows what existing substances are present.

Results: The PCA analysis resulted is RGB images which displayed consistent cellular and extra cellular components with the same color across all data sets (consistent component labelling). Spectral un-mixing results are grayscale images which describe the concentration of various biological components such that a few kinds of nuclei, a few kinds of cytoplasm and a few kinds of collagen were identified. With either method identification of different biological components is made more obvious.

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45. HIGH THROUGHPUT NUCLEIC ACID EXTRACTION AND LIBRARY CONSTRUCTION FROM FFPE TISSUE SPECIMENS

Pandoh P., McDonald H., Corbett R., Smalius D., Tsao P., MacLeod T., Jones M., Kirk H., Merhu S., Ma Y., Coope R., Moore R., Mungall A., Zhao Y., and MARRA M.A.

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We have developed a streamlined approach for the efficient processing of archival formalin-fixed, paraffin-embedded (FFPE) samples. The new workflow is fully automated from FFPE sample extraction to library construction.

With the goal of developing a high throughput automated FFPE sample extraction protocol, a commercially available extraction protocol was evaluated and optimized to work with our liquid handling robot. We customized a protocol for automated total nucleic acid extraction in 96-well format, and validated the process using a variety of FFPE tissue samples.

Improvements were concurrently made to our 96-well FFPE genome library construction protocol, including the substitution of beads-based purification steps for all gel- and column-based steps. In addition, we have implemented a combined FFPE DNA repair and end repair reaction to further streamline the library construction workflow. The increased library yields resulting from these changes made possible a reduction in the DNA input amount requirement. Libraries were successfully constructed from FFPE DNA input amounts ranging from 50 ng to 1 µg.

The combined workflow changes in the GSC’s FFPE Genome sequencing pipeline will facilitate the high throughput processing of large sample sets. A reduction in the library construction input amount also makes the protocol potentially available to smaller samples such as microdissected tissues or small biopsies which are increasingly important for cancer genome studies.

46. EVIDENCE FOR USING PDX MODEL PLATFORM TO ADVANCE PERSONALIZED CANCER MEDICINE

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With the advances of high throughput technologies and the increasing availability of targeted anti-cancer therapeutic agents, personalized cancer medicine is becoming more practical nowadays. Despite many successful case reports, few large cohort clinical trials have been published so far. In this study, we analyzed the evidence-based datasets from these clinical trials and compared them with the data derived from some relevant preclinical datasets which correlate experimental outcomes with the corresponding patients’ clinical outcomes. In addition, we compared the advantages and disadvantages of using subcutaneous vs. sub-renal capsule patient-derived xenograft (PDX) systems. Our preliminary results indicate that the value of using PDX models to implement personalized cancer medicine. These models have the potential to become a key component in the current personalized cancer medicine setting.
47. COMPUTER VISION AS FIRST READER FOR LUNG CANCER SCREENING CT SCANS

Ritchie A.1, Sanghera C.1, Atkar-Khattra S.1, Zhang W.1, Jacobs C.1, van Ginneken B.1, Mayo J.1, Lam S.1, and the Pan-Canadian Early Detection of Lung Cancer Study Group.

Background: The National Lung Screening Trial (NLST) in the United States showed that screening of high-risk individuals with low-dose computed tomography (LDCT) to detect lung cancer early can reduce the lung cancer deaths in high-risk individuals by 20%. The US Preventive Services Task Force (USPSTF) recommends that high-risk individuals aged 55–80 years should receive an annual LDCT. An estimated 1.77 million Canadians and 8.6 million Americans would meet the USPSTF screening criteria. This would have significant resource utilization and quality assurance implications on computed tomography (CT) scanning capacity and interpretation. Computer Vision (CV) software can rapidly screen CT scans for abnormalities to improve screening efficiency and quality assurance. We hypothesize that a trained technician assisted by CV software is able to triage scans that do or do not require a formal reading by radiologists similar to the PAP smear reading model in cervical cancer screening. This approach can potentially save time and money.

Method: A technician with no prior medical experience was trained to operate a CV software (CIRRUS Lung Screening, Nijmegen, Radboud University). This software automatically marks abnormalities detected on screening CT scans. The technician then reviewed a training set of 20 scans processed by the software. After training, the technician reviewed 830 baseline screening CT scans from the Pan-Canadian Early Detection of Lung Cancer Study that were first processed by the software and potential nodules automatically marked without knowledge of the final radiology diagnosis. Within the dataset there were 135 proven cancer cases, 858 cases with benign nodules and 137 cases with no nodules. The technician categorized the scans as normal (does not require formal reading by a radiologist) or abnormal (require a formal reading). All scans were then jointly re-read by two respirologists and re-categorized. The result for both readers was compared to the final radiologist report. For each detected nodule the malignancy risk index was calculated.

Results: All of the cancer cases were correctly categorized as abnormal by the technician. The overall sensitivity of the technician to identify an abnormal scan was very high at 97.8%. The respirologists were comparable, with a sensitivity of 98.1%. The technician identified 87.5% of malignant nodules compared to 94.6% identified by the respirologists (p=NS). The use of the PanCan lung nodule malignancy risk score for scans with nodules identified 127 (22%) of the benign nodule group that potentially not requiring a formal radiologist reading. The average time taken by respirologists and technician to read a scan was 184±85 sec and 208±120 sec respectively (p<0.0001).

Conclusions: A novice technician assisted by CV software correctly categorized abnormal lung cancer screening CT scans for radiology review. Technician specificity may be improved with further training. Pre-screening by a technician may reduce the time taken to review screening CT scans by expert radiologists. The use of CV software with automated calculation of nodule malignancy risk assists case categorization. A combined approach of a trained technician assisted by CV software should improve cost efficiency of CT screening for lung cancer and further investigation is recommended. Computer vision solution enables CT lung cancer screening to be a practical lung cancer control strategy by reducing the workload and improving the speed, consistency and quality of scan interpretation as well as management recommendations among radiologists using an evidence-based approach. The development and implementation of our research is expected to make CT screening practical at the population level.

This work is supported by the Terry Fox Research Institute and the BC Cancer Foundation.

48. MANAGING THE EVOLVING NATURE OF INCIDENTAL FINDINGS IN CLINICAL GENOMICS

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Translating genomic technologies into to the clinical setting has paved the way for doctors and clinicians to apply genomic information for clinical decision-making and treatment. In this study we explore the clinical context of the issues of return of results and incidental findings as a novel genomic test for the life-threatening condition of Acute Myelodysplastic Leukemia (AML) moves into the clinical setting. To examine the opportunities, challenges, and risks to different stakeholders in clinical genomics, we conducted semi-structured interviews with clinicians, clinician-researchers and policy officials. The in-depth, computer-assisted analysis of stakeholder interviews revealed that the most pressing challenges for clinical genomics revolve around the increasingly complex frontiers of returning results and genomic incidental findings. We find a high level of uncertainty and inconsistency among these stakeholders. Many want to return results of genome test. However, there is a wide range of opinions about what type of genome incidental finding (GIF) should or should not be returned. Also, there is no consensus on how and when to return a GIF. This study aims to provide information and analyses that can be used to improve the ethical and procedural guidelines and best practices for clinicians and point of care practitioners managing patient genome information.

This work is supported by the Terry Fox Research Institute. Project title: Improved Assignment to Best Available Therapy for Patients with Myelodysplasia and Acute Myeloid Leukemia (TFRI Project 2018-26).
49. INTRON RETENTION IS WIDELY OBSERVED AND ASSOCIATED WITH INCREASED DNA METHYLATION IN HUMAN CELL TYPES

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Intron retention (IR) is a widely observed but poorly understood mRNA splicing mode where incomplete splicing leads to the retention of intronic sequences from pre-messenger RNAs that can be targeted for degradation by nonsense-mediated decay (NMD). While initially considered to be a result of random errors in the splicing process it has recently been shown to play a role in normal myeloid differentiation suggesting that it is under regulatory epigenetic control and that it might also be involved in the context of blood cancers.

Here we present an IR analysis pipeline developed to identify and characterize IR events in RNA-seq libraries generated from normal and malignant human cell/tissue. Application of this pipeline to a wide collection of human cell/tissue types revealed that the degree of IR correlated with cell type and showed that the blood cell types (CD4 and CD8 naïve and memory primary cells) have the highest degree of IR. Strikingly, we also found that the degree of IR increased with differentiation state across the cell types analyzed and that spliceosomes and SWI/SNF complex members (SMARCA4, SMARCD1...) are down-regulated during cellular differentiation. Epigenetic modifications, like DNA methylation, have been previously correlated with exon usage. Retained introns were found to have increased CpG density overall and we observed a reduction in the degree of 5mCpG change across exon-intron boundaries for retained introns. This observation supports a link between exon-intron boundary DNA methylation and exon splicing which would not be expected to occur at the same degree when the flanking exon is retained. Furthermore in terminally differentiated cell types associated with elevated IR we observed an increase in CpG density for retained introns.

Taken together, our results highlight that intron retention is under epigenetic regulatory control and give new insights in the understanding of epigenetic deregulation in cancer, notably in leukemia.

This work is supported by the Terry Fox Research Institute. Project title: The Terry Fox New Frontiers Program Project in Core Pathogenic Pathways in Human Leukemia.

50. MICRONRNAS: AN ATTRACTIVE BIOLOGICAL SIGNATURE FOR TUMOUR DORMANCY

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3Vancouver Tumour Laboratory (LTL). In this model, the transient expression of miRNAs was investigated in key time points: pre-castration, post-castration (dormant phase), and at the onset of castration-resistant neoplasm. Selecting potential miRNA candidates, we then proceeded to carry out in vitro studies for further validation. Firstly, microarray data showed that the mir-100HG cluster was found to be dramatically up-regulated during the dormant stage (post-castration period) in prostate LTL model (FC: 9.5). This cluster gives rise to three mature miRNAs (mir-let-7a, mir-100, and mir-125b-1*) that have all been reported to have significant roles in cancer biology. Subsequently, qPCR analysis showed that, out of all three mature miRNAs, significant increased expression was observed for mir-100 and mir-125b-1* (FC: 6.0, p<0.0001; FC: 10.0, p<0.0001) during the same dormant stage. Due to sustained increased expression, we then proceeded to carry out in vitro studies on mir-100. However, to address the inadequate models currently available, we developed a novel in vitro model for prostate tumour dormancy. LNCaP cells were grown in media supplemented with androgen-deprived medium or 10% FBS over a period of 2, 4, 6 and 8 days. Upon growth curve analysis, these cancer cells showed absent proliferation while still retaining viability. Collectively, this population of cells demonstrated a key characteristic of dormant tumour cells. In concert with the in vivo data, qPCR analysis showed increased mir-100 expression in androgen-deprived dormant LNCaP cells (FC: 3.5, p<0.0001). Overall, these findings suggest a potential role for mir-100 in tumour dormancy. In future studies, we intend to silence mir-100, determine its effects on dormant tumour cells and, in addition, further characterize our in vitro model for tumour dormancy by carrying out cell-cycle studies.
51. RE-TARGETING VIRUS-SPECIFIC CD8+ T CELLS TO ATTACK TUMOURS

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Introduction: The paucity of known, tumor-specific antigens is an obstacle to the development of targeted cancer immunotherapies. We are developing strategies to overcome this barrier by “in vivo engineering” tumours to express immunogenic foreign antigens. Previously we found that a substantial number of T cells at tumour sites are specific for common viruses such as CMV, EBV, and influenza (CEF). This leads us to hypothesize that by forcing expression of CEF-antigens in cancer cells, it might be possible to re-target anti-CEF immunity toward the tumor. To this end, we are developing an animal model wherein mCMV antigens are expressed in oncolytic viruses (OVs) such as vesicular stomatitis virus (VSV) and vaccinia virus (VV) with cancer-selective tropism. In a complementary approach we are investigating whether pre-existing T cell responses against the OV itself can enhance OV therapy by killing tumour cells that are infected, but do not die from the viral infection.

Methods: To first determine the susceptability of our tumour cell lines to OV infection, we compared the in vitro infectivity of VSV and VV on mouse ID8 ovarian cancer cells and NDP mammary cancer cells. Signs of infection were monitored microscopically over time, and death was quantified with a resazurin-based viability assay. Additionally, we have performed preliminary in vivo experiments with VSV to determine its ability to replicate within a tumour, and whether VSV provides any survival benefit in our models. To address the question of whether pre-existing anti-OV immunity enhances OV therapy, we are vaccinating animals with VSV CD8+ T cell epitopes, treating tumour-bearing animals with VSV, and monitoring T cell infiltration into the tumour and survival.

Results: In both tumour lines, following VSV infection at an MOI of 1, signs of imminent death (cell rounding) were visible 24h post infection at 1:1 concomitant with a 50-80% drop in viability. By 48h p.i. virtually all cells had died. In contrast, VV infection resulted in a 30-50% drop in viability 24h p.i. with no further death as far as 72h p.i. even though cells harbored replicating virus. In vivo, intravenous delivery of VSV resulted in low levels of virus in both ID8 and NDP tumours and marginal improvement in survival. For NDP tumours, intra-tumoural VSV resulted in high levels of virus within the tumour, and slowed tumour growth. For ID8 tumours, intra-peritoneal administration of virus significantly improved survival, however we were unable to detect virus in the majority of tumours. For vaccination experiments, vaccination with VSV peptides induced robust anti-VSV T cell responses. Tumour regression experiments in these animals are currently in progress.

Conclusion and future directions: In vitro experiments suggest that the majority of tumour cells infected with VSV die from direct viral effects. Therefore, providing an additional immune target in the form of mCMV antigens may not yield an additional benefit. However VV- infected tumour cells support high levels of virus replication without dying, suggesting the pre-existing antiviral immunity strategy may be beneficial in the VV model. We are currently optimizing the delivery of VV in tumor-bearing mice, the VV vaccination strategy, and we have cloned mCMV antigens into the VV genome. Future experiments in mice with pre-existing VV or mCMV immunity will assess the ability of VV or recombinant VV-mCMV to cause tumour regression. Success in the animal model would justify steps toward a phase I trial of the concept in humans.

This work is supported by the Terry Fox Research Institute. Project title: Canadian Oncolytic Virus Consortium.

52. EXPLORING EPIGENETIC LANDSCAPES OF RARE PRIMARY HUMAN HEMATOPOIETIC CELL TYPES

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Introduction: Chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq) is a high-throughput technique for comprehensive assessment of histone modifications genome-wide. Current ChIP-seq methodologies require ~1 million cells. This is a major limitation for analyses of many rare primary human cell types such as rare hematopoietic progenitor populations. To circumvent this limitation, some protocols make use of a pre-amplification step prior to library construction. However, the bias introduced into libraries due to pre-amplification restrict their use for comprehensive genome-wide analyses of defined cell populations. Here, we are introducing a ChIP-seq protocol for 10,000 primary human cells. A key element in this alternative protocol was the switch from formamide crosslinking to native ChIP-seq based on mononuclease digestion. To further reduce ChIP-seq variability and allow this protocol to be used in larger scale epigenomic surveys, we have adapted it to an automated liquid handling platform.

Methods: We have used this modified ChIP-seq protocol to profile five histone core markers (Histone3 lysine 4 tri-methylation (H3K4me3), H3K4me1, H3K27me3, H3K36me3, and H3K38me3) of human primary CD34+ cord blood cells. ChIP-seq was conducted on aliquots of 10,000 CD34+ cells purified from cord blood by a 2-step Rosette-Sep EasySep procedure to ≥95% purity and the resulting libraries prepared from the cells were sequenced on an Illumina MiSeq platform in paired-end mode. The resulting sequence reads were aligned by BWA and analyzed using our ChIP-seq analysis tool, FindER.

Results: The resulting enriched regions were compared to Histone marker profiles generated from ~106 CD34+ cells isolated from cord blood. The two datasets show high correlation. For instance, H3K4me3 in two data sets were highly correlated at gene promoters across the whole genome (Pearson =0.96). We also leveraged the nature of the enzymatically digested ChIP libraries to investigate the density of nucleosomes harboring H3K4me3 and H3K27me3 modifications. For this we correlated fragment size (determined by paired-end read boundaries) with ChromHMM-derived chromatin states derived from the CD34+ -NIH reference epigenome. This analysis revealed that the immunoprecipitated fragment sizes correlated with different chromatin states suggesting that these states may be refined by the inclusion of local nucleosome densities.

Conclusions: Our automated ChIP-seq makes it possible to investigate histone modification across the whole genome of rare primary hematopoietic cells with little systematic bias. In addition our assay enables us to simultaneously investigate nucleosome density harboring specific histone modification across the whole genome.

This work is supported by the Terry Fox Research Institute. Project title: Terry Fox New Frontiers Program in Cancer.
Introduction: The pan-Canadian Optically-guided Oral Lesions Trial (COOLS) is a multi-centre randomized control trial designed to measure the effectiveness of a novel tool using fluorescent visualization techniques to draw margins for oral cancer surgeries. As a component of the trial, important health economic questions are being investigated in this population. As the trial reaches its fourth year, many interesting interim results can be reported.

Methods: The trial is collecting three categories of health economic information. The cost of surgical procedures is being collected prospectively through the use of surgical capture forms that record the number and type of staff present for the procedure, as well as equipment used. Second, a questionnaire about costs borne by patients – including travel and accommodation expenses as well as impact on time at work – is being collected at multiple time points over the course of the trial. Third, a set of questionnaires (FACT-HN; EQ-5D; Speech Handicap Index) are being issued to trial participants to assess their self-reported health-related quality of life (QoL).

Results: Descriptive statistics from each of the categories is presented. As recruitment to the trial is ongoing, results are blinded to study arm.

Conclusion: The COOLS Trial is collecting valuable and policy-relevant health economic data concerning the treatment and recovery trajectory of oral cancer patients treated in centers across the country. As we reach full recruitment, we will be able to explore the data comprehensively to examine the health economic implications of oral cancer management in Canada.

This project is funded by the Terry Fox Research Institute.

54. A HIGH-THROUGHPUT METHOD FOR SINGLE-CELL WHOLE-GENOME BISULFITE SEQUENCING

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Functionally and genetically heterogenous cells can exist within a single tumour, which can allow for drug-resistant cells to survive therapy and cause the disease to relapse. Recently, next-generation sequencing has revealed widespread genomic differences between cancer cells from the same tumour. Although tumour heterogeneity is well understood at the genome and transcriptome levels, heterogeneity at the epigenetic level, specifically DNA methylation, has yet to be explored. Whole-genome bisulfite sequencing (WGBS) is a technique used to profile whole genome DNA methylation. However, this methodology is limited by high input DNA requirements and low throughput; therefore, this protocol is not suitable for assessing cancer epigenetic heterogeneity. Thus, we aimed to develop a method that can profile the methylome of rare cell populations, down to single-cells, in order to characterize the epigenetic heterogeneity of tumours and rare cell populations.

We present an improved method, termed Post-Bisulfite Adapter Ligation (PBAL), which combines automated bisulfite conversion of DNA with traditional whole-genome shotgun library construction; this protocol can easily be scaled up with the utilization of a Agilent Bravo liquid handling robot, allowing for interrogation of individual single-cells on a large scale. First, cells are lysed and the genomic DNA is bisulfite converted and purified using magnetic silica beads. Next, the DNA is enzymatically repaired and made into paired-end libraries using a traditional Illumina adapter ligation method. Finally, the libraries are sequenced and aligned to a bisulfite-converted genome using Novoalign.

We successfully generated libraries with an average size of 350 base pairs from 3 different single nuclei from the 184-HTERT L2 cell line. We also successfully generated a library from 180 HCT-116 cells. Furthermore, Agilent Bioanalyzer profiles of the finished library show clear differences between no-template control and single-cells. We aligned the reads using Novoalign and used a filter to remove reads containing many unmethylated cytosines in CHG and CHH contexts. Using this filter, we averaged 1% alignment from no-template controls, 30% alignment from single-cells, and 85% alignment from 100 cells. We saw a 99.5% conversion efficiency of unmethylated cytosines from a control and 1.5% overconversion from a fully methylated DNA spike-in. Analyzing three 184-HTERT single-cells, we saw an average of 15% difference in base-pair CpG methylation.

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MICROTUBULE AFFINITY REGULATING KINASE 2 (MARK2) CONTRIBUTES TO THE MALIGNANT PHENOTYPE OF LUNG TUMOUR CELLS AND IS ASSOCIATED WITH CISPLATIN SENSITIVITY IN NON-SMALL CELL LUNG CANCERS

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Background and Objectives: Non-small cell lung cancers (NSCLC) are a significant cause of cancer-related death in Canada. Advances in understanding the genetic mechanisms of lung tumorigenesis are needed to develop new therapeutic strategies to improve patient prognosis. Using integrative genomic analysis, we identified Microtubule Affinity Regulation Kinase 2 (MARK2) as frequently disrupted by a variety of genetic mechanisms in NSCLC. MARK2 is a serine-threonine protein kinase involved in the regulation of cell cycle and microtubule dynamics associated with Alzheimer’s disease pathology. However, the role of this protein in lung cancer pathogenesis is not known. This work aims to determine whether manipulation of MARK2 expression affects the oncogenicity of NSCLC cells and whether MARK2 is associated with response to the first-line lung cancer chemotherapy, cisplatin.

Methods: MARK2 expression was manipulated in malignant and immortalized non-malignant lung cell lines using lentiviral knockdown and inducible overexpression. Cell viability and anchorage-independent growth assays were conducted to assess the effects of MARK2 expression on oncogenic phenotypes. To identify potential biological pathways modulated by MARK2 in NSCLC cells, pathway analyses were conducted by transcriptomic profiling, luciferase reporter assays, flow cytometry, and Western blotting of MARK2-manipulated cell lines. We also investigated the association of MARK2 with cisplatin sensitivity in lung cancer cell lines.

Results: We determined that knockdown of MARK2 significantly reduced cell viability and colony formation in lung cancer cell lines. Ectopic MARK2 expression in non-malignant lung cells contributed to enhanced cell viability. We found that MARK2 expression was associated with transcription factor activity of many oncogenic pathways, including NF-kappa B. MARK2 expression was negatively associated with NF-kappa B activity and assessment of multiple upstream NF-kappa B pathway proteins suggested MARK2 may be involved in non-canonical NF-kappaB signaling. Additionally, we found that high MARK2 expression was associated with resistance to cisplatin.

Conclusions: We report that changes in the protein expression of MARK2 contribute to oncogenic phenotypes in NSCLC, which are likely mediated through known cancer pathways, including NF-kappaB signaling. Furthermore, we report an association between MARK2 and cisplatin sensitivity, providing evidence of the potential clinical relevance of this protein in NSCLC patient management. Taken together, this study highlights the role of MARK2 in lung cancer pathogenesis, and suggests that further experimentation regarding the biological role of MARK2 in NSCLC is warranted.

This work was supported by the Canadian Institutes of Health Research, National Institutes of Health, the Terry Fox Research Institute, and the BC Cancer Foundation.

BIOINFORMATIC ANALYSES APPROACHES FOR PERSONALIZED ONCOGENOMICS

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The Personalized Oncogenomics initiative at the British Columbia Cancer Agency aims to identify tumor-specific therapeutic targets in cancer patients with late stage disease who have failed standard therapy. Comprehensive profiling of individual patients’ tumors at the DNA and RNA level allows for characterization of altered pathways and hence identification of therapeutics designed to specifically target them.

Data for each individual study included whole genome and transcriptome sequence of the fresh biopsy and whole genome sequence of patient’s blood. When tissues were available, transcriptomic data from a matched normal sample and genome of the commonly formalin-fixed paraffin-embedded primary tumor were also sequenced. All sequencing experiments were performed on Illumina machines. Genomic data was examined, depending on the case, for germline and/or somatic mutations. These included single nucleotide variants, indels and copy number variations. All sequence data were assembled de novo in order to identify rearrangements causing gene fusions; transcriptome data also revealed allelic expression of variants and provided a profile for the entire transcribed genome. Differential abundance estimation was run against a rich repository of publicly available data from The Cancer Genome Atlas project and transcriptome datasets available in-house. The variants and pathways were then mapped to drug databases as well as clinical trial records. This was followed by an extensive literature search for evidence of drug combinations, drug-drug interactions and efficacy of a drug for a particular cancer type, especially those not recognized as the approved disease group for the drug under consideration.

The project has sequenced 50 patients; the average length of time between acquiring the biopsy and delivering a report to clinical oncologists was 37 days. Bioinformatic analysis of the sequence data led to identification of informative or actionable targets in up to 80% of cases. The findings were not restricted to target identification but also led to change of diagnosis, treatment and characterization of tumor evolution. De novo assembly of the data in another patient revealed two divergent and unique tumors. Two different therapeutic were prescribed in order to target these; this led to the disappearance of both tumors and disease stabilization for 7 months. Through the design of an efficient and automated bioinformatics pipeline, individual patient’s tumor specimen was profiled in a clinically relevant time frame. This in turn enabled the delivery of targeted therapies and disease stabilization in patients who had no remaining standard therapeutic options.
57. GENETIC ALTERATIONS OF THE MHC CLASS II TRANSACTIVATOR CIITA ARE FREQUENTLY DETECTED IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA AND ASSOCIATED WITH DIMINISHED MHC CLASS II EXPRESSION

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Introduction: Constitutive MHC class II expression is a hallmark of antigen-presenting cells, including B cells, and is indispensable for the initiation of antigen-specific immune responses. It has been shown that certain B cell lymphoma entities are able to evade immune recognition by downregulation of MHC molecules on the tumor cell surface. We have previously identified recurrent chromosomal rearrangements and somatic mutations of CIITA, the master regulator of MHC class II transcription, as one possible mechanism to reduce MHC class II expression in primary mediastinal large B-cell lymphoma (PMBCL) and classical Hodgkin lymphoma (cHL). Therefore, we aimed to explore the frequency of these alterations and the correlation with CIITA and MHC class II protein expression in a larger cohort of PMBCL cases and to further characterize their functional significance.

Methods: We have comprehensively analyzed 45 diagnostic PMBCL samples for the presence of coding sequence mutations as well as alterations within the promoter III region and the first 3kb of intron 1 using deep amplicon sequencing (Illumina TruSeq) and/or Sanger sequencing. In addition, we characterized the PMBCL-derived cell lines U2940 and Med-B1 by whole transcriptome paired-end sequencing (RNA-seq). To elucidate the functional consequences we performed retroviral transductions of wild type CIITA and CIITA mutants in the CIITA and HLA-DR expression-negative cell line DEV. We subsequently analyzed CIITA mRNA expression using qRT-PCR and HLA-DR expression using flow cytometry. Furthermore, we applied immunohistochemistry (IHC) to determine expression levels of CIITA and HLA-DR in a large cohort of PMBCL samples represented on tissue microarrays.

Results: Within the subset of 45 PMBCL cases that were analyzed for the presence of genomic alterations, 39% were CIITA-ba (16/41) and in 31.8% (14/44) we observed coding sequence mutations and/or alterations affecting the promoter III region. 45.5% (20/44) of the cases presented indels and/or SNVs in intron 1. Using RNA-seq, we have detected two missense mutations in functionally relevant protein domains in the Med-B1 cell line. Furthermore, we identified a novel NUBP1-CIITA fusion in U2940 also harboring an SNV on the other allele resulting in the transcription of an elongated protein due to the loss of the original stop codon. Ecotropic expression of these CIITA mutants in DEV revealed that these individual SNVs showed a diminished capability to restore HLA-DR surface expression in comparison to wild type CIITA as measured by flow cytometry.

Correlative analyses revealed that decreased CIITA-protein expression by IHC was significantly correlated with the presence of CIITA-ba (P=0.019), whereas HLA-DR expression was not correlated with CIITA-ba status alone (P=0.219). However, we could demonstrate a positive correlation between protein expression of CIITA and HLA-DR (Pearson r=0.45, P<0.0001).

Conclusions: Here we show that CIITA is frequently targeted by coding sequence mutations and intronic deletions in PMBCL cell lines and clinical samples. Functional studies demonstrate that genomic alterations in CIITA contribute to downregulation of MHC class II expression in malignant lymphomas and therefore represent a potent mechanism of acquired immune privilege and escape from immune surveillance.

58. ENABLING QUANTITATIVE DIGITAL PATHOLOGY FOR WHOLE SLIDE SCANNERS: PROSTATE CANCER AND ORAL DYSPLASIA PROGNOSIS APPLICATIONS


Cancer is the leading cause of death in North America and responsible for 30% of all deaths. Cancer is most likely curable if it is detected at early stages. In this study we plan to develop new methods for early cancer detection for two types of cancer, prostate and oral. Although, current Prostate Cancer (PCA) screening methods are effective in early cancer detection; treatment following that can affect life quality. Also a major barrier to oral cancer prevention is the inability to detect oral premalignant lesions. To increase the chance of cancer prevention, developing new techniques for cancer diagnosis/prognosis is highly desirable.

Our goal is to develop tools for automated, and cost-effective Digital Pathology (DP) to obtain clinically relevant data from digital images. We will then use image analysis/interpretation to detect anomalies in cell structure. We will also design a quick and simple method for evolution and correction of the imaging system to ensure quality assurance. Ultimately, we plan to develop, test, and validate the DP methodology and estimate its performance characteristics for the prediction of cancer (PCa and Oral) progression risk or recurrence from sectioned tissue material.
59. VARIOMIC ANALYSIS OF PARP INHIBITOR SENSITIVITY USING THE C. ELEGANS MILLION MUTATION PROJECT STRAINS AND HUMAN CELL CULTURES IDENTIFIES NEW PARPI-SENSITIVE GENOTYPES

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Poly ADP-ribose polymerase (PARP) inhibitors are excellent examples of anti-cancer therapeutics that exploit synthetic lethality to affect tumour killing. PARP inhibitors have been shown to kill cells defective in BRCA1/BRCA2-mediated homologous recombination (HR) repair both in cell culture and in clinical trials. Although PARP inhibitors show promise as potent anti-tumour therapeutics with high selectivity for killing tumour cells with relatively few side-effects, several questions need to be addressed to fully realize the therapeutic potential of PARP inhibitors. How do PARP inhibitors affect genotype-specific tumour cell killing and what is the full spectrum of tumour genotypes that are sensitive to PARP inhibition? What is needed is a fundamental understanding of the role of PARP in the DNA damage response and a comprehensive catalog of gene mutations that are synthetic lethal or synthetic cytotoxic with PARP inhibitors. We are using the model organism Caenorhabditis elegans as an in vivo “variomics” resource to investigate the gene mutation spectrum of synthetic lethality and synthetic cytotoxicity with PARP inhibition.

Previous work in our lab extended the repertoire of genotypes that are sensitive to PARPi by demonstrating that PARP inhibition is synthetic lethal with mutations affecting cohesin genes, which are frequently mutated in a number of tumour types. We initially discovered this interaction in C. elegans and later showed that it was conserved in cultured human colorectal and glioblastoma cell lines. Recently, a large-scale C. elegans variomics resource, the Million-Modification Project (MMP) strain collection, has become available that has allowed us to screen for PARPi-sensitive genotypes in a high throughput manner. The MMP collection of 2,007 mutagenized and sequenced C. elegans lines contains >800,000 mutations with >183,000 non-synonymous changes predicted to affect ~19,000 proteins. As part of a Canadian Cancer Society Research Institute-funded Innovation grant, we conducted a feasibility screen with ~400 strains (~40,000 mutations) and found ten strains sensitive to PARPi inhibition. Frequency analysis of the mutations in these ten strains revealed mutations affecting three genes that are likely responsible for the PARPi-sensitivity in seven of the ten strains. Mutations in these genes were not previously shown to be sensitive to PARPi inhibitors. Detailed analysis of the mechanism underlying the synthetic lethality of one of the SL partners in human cell culture has demonstrated that this interaction is conserved and is phenotypically different than the lethality observed in HR-deficient cells treated with PARPi inhibitors. This variomic screening approach has potential to discover new clinically relevant SL interactions and has expanded our understanding of the role PARP in cellular pathways.

60. EXAMINATION OF THE ROLE OF ENDE IN HUMAN CANCER

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In 2010, our lab identified Ende, a short secreted peptide enriched in the definitive endoderm of mouse embryo. Recently, two independent groups have discovered in zebrafish that Ende is a novel ligand for the APJ receptor and it is required for both cardiac formation and endoderm development. However, little is known about the role of Ende in humans.

Human ENDE has been reported to be highly expressed in human embryonic stem cells (hESC) and down-regulated rapidly during HESC differentiation. Gene expression datasets show low expression levels in normal tissues but up-regulation in certain ovarian tumour samples. In addition, the Cancer Cell Line Encyclopedia (CCLE) database affirms that ENDE is highly expressed in Ewing’s sarcoma and two ovarian cancer cell lines. RT-PCR confirmed that several Ewing’s sarcoma and ovarian cancer cell lines express ENDE. Taken together, ENDE has a potential pathological role in human cancer.

The APJ receptor signals through various G-coupled proteins, activating PKC/RAS/MEK/ERK and PI3K/Akt pathways and inhibiting forskolin-stimulated production of cAMP. Our preliminary data showed that ENDE can also stimulate phosphoERK and phosphoAKT, suggesting ENDE potentially functions through APJ receptor signalling. Thus, the hypothesis is that human ENDE regulates cell migration and growth in ovarian cancers and Ewing’s sarcomas by binding the APJ receptor and activating its signalling.

Functional studies will focus on cell lines with varied levels of ENDE expression. We will examine how altering ENDE expression, by either knocking it out with CRISPR or overexpressing it using a lentiviral expression system, will impact basic cell functions, such as cell migration and proliferation. We are currently generating antibodies specific to ENDE to evaluate the correlation between ENDE expression and patient outcomes by IHC staining on tumour Tissue Microarray (TMA).

The field of Ende research remains mostly unexplored. Our studies may provide the molecular foundations for the potential of ENDE as a therapeutic peptide target for the prevention of tumour growth.

This work was supported by funding from Genome British Columbia, Genome Canada and Canadian Institutes of Health Research, and Natural Sciences and Engineering Research Council of Canada.
61. DEFINE: A NOVEL APPROACH TO NORMALIZATION FREE DIFFERENTIAL EXPRESSION DETECTION

Bilenky M.1, Zhang W.1, Carles A.2, Jones S.J.M.1, HIRST M.1,2.

1Canada’s Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada; 2Department of Microbiology and Immunology, Centre for High-Throughput Biology, University of British Columbia, Vancouver, BC, Canada.

Analysis of messenger RNA and proteins is widely used to compare patterns of gene expression between cells or tissues of different kinds and under different conditions; for example, between normal and cancer cells. Here we present a novel analytical tool to detect differential gene expression patterns from deep next generation sequencing RNA-seq data.

A number of software packages were developed in recent years to predict differentially expressed genes. The challenges for majority of these tools is a choice of global normalization as well as a selection of the shape of the expression distribution, which is a priori unknown.

In the proposed approach, we attempted to minimize effects of both uncertainties mentioned above. Our tool, called DEfine, finds genes that are significantly differentially expressed between two data sets without requirement of absolute normalization. First, DEfine analyses and corrects, if necessarily, apparent biases in the data such as dependence of the signal on the GC content of the mRNA sequence, or the length of the gene. After that the background distribution is inferred from the data and the outliers are detected. The p-values are assigned and FDR controlling procedure is applied before the final thresholding. DEfine incorporates mRNA spike-in control data, if it is available for the analyzed data sets. DEfine is built upon outputs of our existing extensive RNA-seq QC and analysis pipeline.

DEfine is currently written in Matlab and Java and outputs lists of differentially expressed genes as well as several publication quality plots that help to visualize the comparative analysis. We illustrate an applicability of the DEfine on the recent strand-specific RNA-sequencing data, in particular for mammary gland epithelial cells.

This work is supported by the Terry Fox Research Institute. Project title: The Terry Fox New Frontiers Program Project in Core Pathogenic Pathways in Human Leukemia.

62. RCOR1 LOSS AND FUNCTIONAL CHARACTERIZATION IN DIFFUSE LARGE B CELL LYMPHOMA

Healy S.1, Chan F.C.1,2, Telenius A.1,2, and STEIDL C.1

1Centre for Lymphoid Cancer, BC Cancer Agency, Vancouver, BC, Canada; 2Bioinformatics Graduate Program, University of British Columbia, Vancouver, BC, Canada

RCOR1 is a co-repressor, a component of a large multiprotein complex including REST, HDAC1/2 and LSD1 that regulates gene expression in a variety of biological contexts. We have recently found following integration of high-resolution genotyping arrays and RNA-Seq data of 148 genomes with 91 matching transcriptomes that RCOR1 is deleted (n = 11; 7.5%) in a subgroup of diffuse large B cell lymphoma (DLBCL) patients. RCOR1, though well described in neural and hematopoietic differentiation, has not been implicated in carcinogenesis, making this discovery the first of its kind. This novel deletion correlates with poorer prognosis in R-CHOP (standard therapeutic administered to DLBCL patients) treated patients. A gene expression signature of 233 genes, including integration of data from stable RCOR1 shRNA knockdown in Raji and KM-H2, a Burkitt and Hodgkin lymphoma cell line, respectively, was found to be significantly associated with inferior survival in these patients. This gene expression signature was further categorized and ranked into common cellular pathways using pathway enrichment analysis. A selection of downregulated genes within these correlative pathways was further validated in Raji cell lines following RCOR1 knockdown or biallelic knockout using the CRISPR method. We found from this directed study a significant downregulation of a number of pro-apoptotic genes in cells with reduced RCOR1. This suggests an upstream role for RCOR1 in regulation of apoptosis, indicating a pro-survival phenotype in cells with reduced RCOR1 expression. As such, we proposed a novel role for RCOR1 in tumorigenesis and decreased sensitivity to standard chemotherapy. These studies introduce a novel high-risk subgroup of DLBCL, defining a new course for therapeutic intervention.
These results show that there is the potential for serum miRNAs to act as a biomarker for oral cancer. Individuals with oral cancer have a poor survival rate and a high level of recurrence due mainly to the late stage of diagnosis. New methods are required in order to increase survival rates. miRNAs are a group of small nucleotide molecules, involved in gene regulation that have been linked to tumour suppressing and oncogenic roles in cancer. Circulating miRNA expression profiles have been shown to be useful in delineating healthy individuals from those with various types of cancer.

Objective: To determine the ability of serum miRNAs to act as a biomarker for oral squamous cell carcinoma.

Methods: Serum was collected from patients with oral squamous cell carcinoma (OSCC) and oral carcinoma in situ (CIS) as well as demographically matched non-cancer controls. RNA extracted from the serum samples was profiled using miRCURY LNA Universal RT miRNA PCR panels to assess the expression of 742 miRNAs. miRNAs known to be affected by haemolysis in blood samples were excluded from analysis. A model to distinguish between OSCC/CIS and non cancer individuals was created using logistic regression on miRNAs included in the model by LASSO analysis, a method that preferentially creates statistical models with fewer miRNAs.

Results: By performing statistical analysis on our samples after they had been randomly split into training and test sets we were able to determine that our model was able to achieve a higher than 80% accuracy in differentiating between cancer and control samples. By including 18 miRNAs in our model/biomarker we were able to achieve a higher than 80% accuracy in differentiating between cancer and control samples by including a pair of 17-gestational-week monozygotic twins. Differential methylation analysis identified on average 255 genes with promoter cortex UMRs, and 456 GE UMRs including paired box 6 (PAX6). Further transcriptional analysis identified 382 cortex up-regulated genes and 456 GE up-regulated genes shared by at least two individuals. Both were enriched in neuronal development and cell migration. DNA methylation profiling at exon boundaries showed a general correlation between mCpG and exon usage, but no significant difference between cortex and GE for cell-type specific exons. Taken together, our results suggest mCpG provide a stable methylation profiling at exon boundaries showed a general correlation between mCpG and exon usage, but no significant difference between cortex and GE for cell-type specific exons. Taken together, our results suggest mCpG provide a stable.
65. DETECTION OF RECURRENT MUTATIONS FOR RISK STRATIFICATION IN ACUTE MYELOID LEUKEMIA

Pilsworth J.1,2, Chiu R.1, Nip K.M.1, Bohsaz B.1, Docking T.R.1,2, Karsan A.1,4, BIROL I.1,2.

1Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, Canada; 2Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 3Department of Medicine, University of British Columbia, Vancouver, Canada; 4Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada.

Acute myeloid leukemia (AML) is a genetically heterogeneous disease characterized by the accumulation of acquired somatic genetic mutations in hematopoietic progenitor cells. With current chemotherapy regimens the survival rate is 35-40% for patients under the age of 60 and 10-15% for patients over the age of 60. The discovery of fusion proteins and molecular alterations, such as PML-RARγ and internal tandem duplications in FLT3, aids in classifying the molecular genetic heterogeneity within cytogenetically defined subsets of AML. Detection and characterization of such recurrent genetic mutations has led to the development of risk-stratified approaches.

Here, we report on the results of Trans-ABySS, an assembly and analysis tool that detects structural variations and genetic alterations that are important for risk stratification in AML. We performed transcriptome shotgun sequencing experiments on a cohort of 184 AML patients. I used Trans-ABySS to assemble and analyze the transcriptomes of a subset of these AML patients with specific clinical classifications. My results demonstrate that our analysis pipeline consistently identifies clinically relevant markers such as fusion events between PML and RARγ, fusion events between MYH11 and CBFγ, internal tandem duplication events in FLT3, partial tandem duplication events in MLL, and an insertion in NPM1. Moreover, Trans-ABySS detected novel mutation events in the selected subset of AML patients. To determine the prevalence of these novel events, I used the breakpoint sequences from the assemblies to probe against the AML transcriptomes as well as different cancer transcriptomes, for example, from The Cancer Genome Atlas (TCGA) project.

66. COLORECTAL CELLS LACKING THE TUMOUR SUPPRESSOR GENE FBW7 REQUIRE AN INTACT SPINDLE ASSEMBLY CHECKPOINT

Bailey M., Singh T., and HIETER P.

Michael Smith Laboratories, University of British Columbia, Vancouver, BC.

FBW7 (F-box and WD repeat domain-containing 7), also known as hCDC4, is a tumour suppressor gene mutated in a broad spectrum of cancer cell types, most notably in more than 8% of colorectal carcinomas. As a component of the SCF E3 ubiquitin ligase, FBW7 is responsible for specifically recognizing a broad spectrum of phosphorylated substrates and targeting them for ubiquitin-mediated degradation. Many FBW7-targeted substrates have known roles in various areas of tumour development and progression including proliferation, apoptosis, hypoxia, metastasis, chromosome instability (CIN) and chemotherapeutic drug resistance. Although the role of FBW7 as a tumour suppressor is well-established, less well-studied is how FBW7-mutated cancer cells might be targeted therapeutically. To explore this further, we undertook a genome-wide RNAi screen using X4T and FBW7 knockout colorectal cell lines and identified the spindle assembly checkpoint (SAC) protein BUBR1, as a candidate genetic interaction target.

Loss of FBW7 has previously been linked to defective mitotic progression and CIN. We show here that asynchronous FBW7 knockout cells have increased levels of mitotic APC/C targets and are vulnerable to knockdown of BUBR1 and other SAC components, suggesting a dependence of these cells on the mitotic checkpoint. Knockdown of BUBR1 with RNAi can override mitotic arrest and resulted in significant cell polyploidy. Analysis of FBW7 substrates suggested that the cell cycle component Cyclin E was necessary, but not sufficient, for this genetic interaction. Finally, introduction of cancer-specific FBW7 mutations into cells demonstrated that they also deregulate Cyclin E and show characteristics of CIN similar to FBW7 knockout cells. Our work shows that the CIN of FBW7 knockout cells is a vulnerable phenotype for potential therapeutics, especially those that target the spindle assembly checkpoint.
Although there is minimal recurrence of SNVs in individual genes, some cancer-related genes not previously understood paediatric bone cancer osteosarcoma and particularly to understand its propensity to metastasize. These data are being analyzed to investigate driver genes and biological pathways implicated in its formation and metastasis and to better delineate the patterns of clonal evolution in this malignancy.

Materials and methods: We performed whole genome and deep exome sequencing on the primary tumour and matched normal tissue samples from 13 osteosarcoma patients in addition to ten paired metastases and three chemotherapy-treated primary tumours. We similarly sequenced 23 primary tumour and metastasis samples (without matched normal tissue), which were collected from a separate cohort of 12 patients to aid in identifying recurrent alterations. This unique dataset provides the basis for the comprehensive description of the mutational landscape in osteosarcoma and will facilitate the identification of recurrent genomic alterations involved in metastasis. We are integrating a number of computational methods to maximize the biologically relevant information that can be extracted from the data.

Results: Although there is minimal recurrence of SNVs in individual genes, some cancer-related genes not previously linked to osteosarcoma were found genetically altered. Notably, a known hotspot mutation in the oncogene GNAQ was detected in one patient, and a potential kinase-activating mutation in KDR (VEGFR2) was found in another. When both SNVs and CNVs are considered together, the calcium signalling and angiogenesis pathways are significantly altered. The relative overlap in terms of SNVs between matched primary tumours and metastases was lower than expected, being on average 8.7 %, highlighting the heterogeneity of these tumours and the possibility of early metastatic dissemination followed by ongoing somatic mutation.

Moreover, SVs are thought to play a crucial early role in osteosarcoma oncogenesis. Several genes were found to be structurally rearranged in a number of patients, many of which have been associated with cancer. Characterization of these rearrangements is ongoing. This includes comparing the clonality of SVs as well as SNVs between matched primary tumours and metastases. This analysis will serve to elucidate the clonal evolution that took place during osteosarcoma formation and metastasis, and thus help determine drivers of tumourigenesis.

Conclusions: The identification of genetic events common to matched primary tumours and metastases and those unique to metastases will potentially reveal novel driver genes or biological pathways causally implicated in osteosarcoma oncogenesis and metastasis, respectively. This knowledge may ultimately be translated to the clinic by informing the development of improved personalized treatments and diagnostic biomarkers.

References:

Materials and methods: We are using genome and exome sequencing to characterize the molecular aetiology of the poorly understood paediatric bone cancer osteosarcoma and particularly to understand its propensity to metastasize. These data are being analyzed to investigate driver genes and biological pathways implicated in its formation and metastasis and to better delineate the patterns of clonal evolution in this malignancy.

Results: Although there is minimal recurrence of SNVs in individual genes, some cancer-related genes not previously linked to osteosarcoma were found genetically altered. Notably, a known hotspot mutation in the oncogene GNAQ was detected in one patient, and a potential kinase-activating mutation in KDR (VEGFR2) was found in another. When both SNVs and CNVs are considered together, the calcium signalling and angiogenesis pathways are significantly altered. The relative overlap in terms of SNVs between matched primary tumours and metastases was lower than expected, being on average 8.7 %, highlighting the heterogeneity of these tumours and the possibility of early metastatic dissemination followed by ongoing somatic mutation.

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Conclusions: The identification of genetic events common to matched primary tumours and metastases and those unique to metastases will potentially reveal novel driver genes or biological pathways causally implicated in osteosarcoma oncogenesis and metastasis, respectively. This knowledge may ultimately be translated to the clinic by informing the development of improved personalized treatments and diagnostic biomarkers.

References:


68. TRAF3 INACTIVATION IN CANINE AND HUMAN B-CELL LYMPHOMA

Bushnell K.R.1, Kim Y.1, Chan F.C.3,3, Ben-Neriah S.1, Jenks A.1, Alcaide M.1, Fornika D.1, Grande B.1, Gascoyne R.D.3, Steidl C.1, and MORIN R.D.12.

Background: Non-Hodgkin lymphomas (NHLs) are the fifth most common cancer in humans and the most common cancer to affect pet dogs. Dogs hold promise as clinical models for human cancers due to their propensity to spontaneously develop tumours at a rate comparable to humans. In contrast to the many genes whose mutation contributes to lymphomagenesis in humans, relatively little is known about the acquired genetic alterations harboured in canine NHLs. In order for a canine model of human malignancy to be realized, the genetic commonalities and differences between diseases in the two species must be understood. We surveyed 65 canine B-cell lymphomas (cBCLs) with matched normal tissue samples to identify genes significantly affected in this disease. We found a single gene, TRAF3, recurrently mutated in cBCL and confirmed TRAF3 inactivation also occurs in human diffuse large B-cell lymphomas (hDLBCL).

Methods: Fourteen cBCLs tumours were analyzed with a combination of RNA-seq and exome sequencing to screen for recurrently mutated genes. The coding exons of TRAF3 were also sequenced in an additional 46 cases. Deep-amplicon sequencing was performed on the tumours and matched normal to validate all putative mutations and confirm their origin as germline or somatic. Copy number and gene expression analysis was performed on a cohort of 148 hDLBCLs.

Results: We found mutations affecting TRAF3, which encodes a negative regulator of NF-κB activity, in 9% of human DLBCLs and reduced expression of TRAF3 among deleted cases. Notably, a known hotspot mutation in the oncogene GNAQ was recurrently mutated in cBCL and confirmed TRAF3 inactivation also occurs in human diffuse large B-cell lymphomas (hDLBCL). Deregulation of NF-κB activity is a known characteristic of the more aggressive activated B-cell (ABC) subgroup of DLBCL. This study implicates mutations affecting NF-κB activity as a novel genetic commonality between human and canine NHLs and supports the potential utility of cBCLs with mutated TRAF3 as a model of the ABC-DLBCL.
To describe the heterogeneity associated with GATA2 mutation and highlight features that should prompt early recognition. Although the literature is biased towards individuals who present with overt hematological malignancies, EMBERGER syndrome is caused by mutation in GATA2 and predisposes to myelodysplastic syndrome (MDS)/acute myelogenous leukemia (AML), lymphedema, warts, subtle dysmorphic features, and, rarely, congenital anomalies.

We have recently developed treatment resistant cell line models and identified that our low PSA expressing prostate cancer cells have cancer stem cell characteristics. To define molecular mechanisms contributing to treatment resistance, we performed gene profiling on our prostate cancer stem cells expressing low levels of PSA. Using systems biological approaches, we found that several genes and pathways involved in stem cell maintenance were altered. Interestingly, forkhead box M1 (FOXM1) was one of the main pathways potentially maintaining cancer stem cells and driving dedifferentiation during treatment resistance. FOXM1 is a transcription factor that confers treatment resistance and is involved in maintenance of pluripotency in stem cells. FOXM1 also correlates poor survival in prostate cancer patients. Using systems biological approaches, we identified novel potential FOXM1 inhibitors reversing the gene expression signature of our cells. We further validated the effect of these compounds in our prostate cancer stem cells and identified VPCKK as a novel inhibitor FOXM1. The results indicated that VPCKK binds to FOXM1 and reduces the cell viability, FOXM1 transcriptional activity as well as FOXM1 protein, gene and its target gene expressions in our prostate cancer stem cells.

Taken together, the results suggest that inhibition of FOXM1 by VPCKK is a potent novel mean to target low PSA expressing prostate cancer stem cell-like cells resistant to current therapies.

Acknowledgement: Prostate Cancer Canada Movember and Terry Fox Research Institute. The title of research project that has conducted the research described: “Rationale targeting developmental pathway in Enzalutamide resistance”, “Defining the role of Lyn kinase in prostate cancer progression to castrate resistant stage”.

Background: EMBERGER syndrome is caused by mutation in GATA2 and predisposes to myelodysplastic syndrome (MDS)/acute myelogenous leukemia (AML), lymphedema, warts, subtle dysmorphic features, and, rarely, congenital anomalies.

Objectives: To describe the heterogeneity associated with GATA2 mutation and highlight features that should prompt testing; to discuss suggested surveillance guidelines and timing of intervention in individuals found to carry a GATA2 mutation.

Design/Methods: Case report of 2 families with mutation confirmed EMBERGER syndrome and literature review of treatment and surveillance guidelines.

Case Report – Family 1: The proband presented with warts and mouth ulcers. WHIM syndrome was considered when neutropenia and B cell deficiency developed, despite negative CXCR4 testing. Years later, he developed AML with monosomy 7 and underwent chemotherapy and stem cell transplantation. Idiopathic leg lymphedema occurred. Family history was significant for warts. Re-examination revealed subtle dysmorphic features. Mutation analysis of GATA2 detected a missense mutation.

Family 2: Sibling 1 had a history of warts, mouth ulcers, vesicoureteral reflux (VUR), ectopic anus, mild sensorineural hearing loss, and new-onset neutropenia. Immunological testing identified several abnormalities. Bone marrow testing diagnosed MDS with monosomy 7. Sibling 2 was found to have hypocellular, mildly dysplastic bone marrow. History included warts, VUR, and leg lymphedema. Sibling 3 had mild bone marrow hypocellularity. Paternal history was significant for childhood warts and mouth ulcers. Siblings 1-3 had subtle dysmorphisms. GATA2 sequencing identified a nonsense mutation. Sibling 1 underwent stem cell transplantation. Siblings 2-3 are closely monitored.

Literature review: Management of individuals carrying a GATA2 mutation is complicated by inter- and intra-familial variability. However, suggested surveillance guidelines include regular clinical examination, CBC with differential, and bone marrow biopsy, as well as ongoing consideration for stem cell transplantation. Intervention before development of AML decreases morbidity/mortality.

Conclusions: Although the literature is biased towards individuals who present with overt hematological malignancies, EMBERGER syndrome can be recognized earlier. Suspicion should be high in patients with persistent hematologic/immunologic abnormalities, warts unusually refractory to treatment, and/or lymphedema, particularly in the setting of subtle, but typical, dysmorphisms.
71. PEDIATRIC PERSONALIZED ONCOGENOMICS (PedsPOG) – INITIAL OUTCOMES

Conclusions: The initial pilot project into pediatric personalized oncogenomics has yielded interesting findings in a significant proportion of those enrolled and has resulted in therapeutic decisions in three of the seven enrolled (with one subject of the seven pending). Ongoing cases are being recruited into the study.

72. THE BURDEN OF CO-MORBIDITY ON POST-SURGICAL ADVERSE EVENTS IN EARLY-STAGED ORAL CANCER TREATMENT

Conclusions: The presence of co-morbidity indeed has significant impact on the adverse events post treatment of early-staged oral cancer patients.


References:
1. RASSEKH S.R.
2. DEYELL R.
3. Lee A.
4. Dunham C.
5. Virani A.
6. Jantzen C.
7. LASKIN J.
8. MARRA M.

Purpose: Utilization of genomic information in order to augment chemotherapy decision making in children with relapsed or refractory tumors.

Methods: Children with relapsed or refractory malignancies were identified by their treating oncologist at a single institution (British Columbia’s Children’s Hospital). After full informed consent (and assent where applicable), a biopsy was collected either at the time of clinical biopsy or specifically for enrollment on this study. Peripheral blood was collected for germline testing, as well as for identification of circulating tumor DNA. Tumor was biopsied either by general surgeons or by interventional radiology and processed by the Genome Sciences Center. Original tumors (fresh frozen tissue where possible, if not then FFPE) were identified in the pathology archives and also processed by the Genome Sciences Center. Whole genome sequencing, transcriptome sequencing and RNA sequencing was performed. Each patient’s tumour genomics data was compared to the literature and to a comprehensive database of drugs with the aim of identifying drugs most likely to target individual tumors based on the presence of mutated or aberrantly expressed genes in the tumor. All cases were reviewed at a weekly tumor board attended by both scientists and clinicians and treatment options were discussed. Therapies were ultimately decided upon by the treating oncologist in conjunction with the patient and family.

Results: Nine subjects have been approached for enrollment into this trial and 7 subjects have been enrolled (3 with neuroblastoma, 1 CNS sarcoma, 1 infantile fibrosarcoma, 1 pleomorphic neurifibroma, and 1 with metastatic melanoma). In 5 of the 7 subjects viable tumor tissue was biopsied and genomic results are available. In 2 subjects, no viable tumor was identified in the sample and one of these has subsequently been re-biopsied with successful collection of tumor. Results of genotyping not yet available for this subject. In 3 subjects novel translocations were identified. All 3 of these subjects also had targetable pathways identified and were started on therapies due to results from PedsPOG. Two subjects were started on ALK inhibitors (crizotinib) and one subject was started on a JAK inhibitor (ruxolitinib). The adolescent with infantile fibrosarcoma has a partial response to therapy that was sustained for 9 months. The patient with neuroblastoma had stable disease for a brief period of time prior to relapse, and the child with CNS sarcoma has had marked clinical improvement on JAK inhibition with stable disease on imaging (6 weeks into therapy).

Methods: To up to the end of June, we have 322 (89%) patients had at least 3 months post-surgery follow-up visits to complete the adverse event. Among the initial analysis on 298 patients, the majority were at average age of 61.7±13.2 years, male (63.6%), ever-smokers (68.5%), and having lesions on the tongue or floor of mouth (82.6%). In addition to intratrual tumor resection, some received additional neck dissection (28.5%) and/or adjuvant radiotherapy (14.8%). Of the 298 patients, 188 (66.4%) presented with comorbidity of ACE grade 1 or above with hypertension (39%) and diabetes mellitus (13%) on the top of the most common occurring conditions. Thirty-three (10.7%) patients reported with AEs within 3 months of surgery. Using ANDVAA, only anatomical site was associated with AEs (P = 0.03). In order to examine the effect from the type of the comorbid conditions, using multivariate logistic regression analysis with the adjustment of age and anatomical site, conditions (history of coronary heart disease, congestive heart failure, hypertension or arrhythmia) in the cardiovascular system showed significant impact on the presence of greater or equal to grade 3 AEs with 5 times elevated risk comparing those without, respectively (P = 0.002).

Purpose: To up to end of May 2014, the COOLS trial has completed the recruitment of 400 patients from 7 participating sites across Canada. The co-morbid status of the patients prior to surgery was evaluated using the validated Adult Co-morbidity Evaluation-27 (ACE) to record the type and severity. Other relevant clinic-pathological variables (age, gender, smoking history, anatomical site, presence of nodal disease, and additional treatment modality) were retrieved. Univariate and multivariate logistic regression analyses were used to evaluate the effect of these variables on the presence of Grade 3 and above adverse events (AE) within 3 months post surgery.

Results: To up to the end of June, we have 322 (88%) patients had at least 3 months post-surgery follow-up visits to complete the adverse event. Among the initial analysis on 298 patients, the majority were at average age of 61.7±13.2 years, male (63.6%), ever-smokers (68.5%), and having lesions on the tongue or floor of mouth (82.6%). In addition to intratrual tumor resection, some received additional neck dissection (28.5%) and/or adjuvant radiotherapy (14.8%). Of the 298 patients, 188 (66.4%) presented with comorbidity of ACE grade 1 or above with hypertension (39%) and diabetes mellitus (13%) on the top of the most common occurring conditions. Thirty-three (10.7%) patients reported with AEs within 3 months of surgery. Using ANDVAA, only anatomical site was associated with AEs (P = 0.03). In order to examine the effect from the type of the comorbid conditions, using multivariate logistic regression analysis with the adjustment of age and anatomical site, conditions (history of coronary heart disease, congestive heart failure, hypertension or arrhythmia) in the cardiovascular system showed significant impact on the presence of greater or equal to grade 3 AEs with 5 times elevated risk comparing those without, respectively (P = 0.002).

Conclusion: The presence of co-morbidity indeed has significant impact on the adverse events post treatment of early-staged oral cancer patients.

# Terry Fox BC-Funded Projects*

*As of press time, October 15, 2014. Listed projects were active in 2014 calendar year and forward.

The above includes total funding from TFRI and its partners for the BC-funded portion of the projects.

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<td>2012-2015</td>
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*As of press time, October 15, 2014. Listed projects were active in 2014 calendar year and forward. The above includes total funding from TFRI and its partners for the BC-funded portion of the projects.
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More details coming soon www.tfri.ca/asm

**Where:** St. John’s, Newfoundland & Labrador (Delta St. John’s Hotel and Conference Centre)

**Program and Session Dates:**
Thursday, May 7th and Friday, May 8th

**Suggested Travel Dates:**
Arrival – Wednesday, May 6th
Departure – Saturday, May 9th

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**Our Collaborative Partners**

**TERRY FOX MARATHON OF HOPE • 1980-2015**

*Terry Fox at the ocean edge, Newfoundland, 1980*

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**A single dream. A world of hope.**

*The Terry Fox Foundation*

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**CARE + RESEARCH**

*BC Cancer Agency*

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**partners in discovery**

*BC Cancer Foundation*

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**Healthier lives through discovery**

*Vancouver Coastal Health Research Institute*

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**Transforming Discovery into Opportunity**

*cdrd, The Centre for Drug Research and Development*

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**How you want to be treated.**

*Providence Health Care*

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**Network partners.**

*SFU, Team Finn*

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