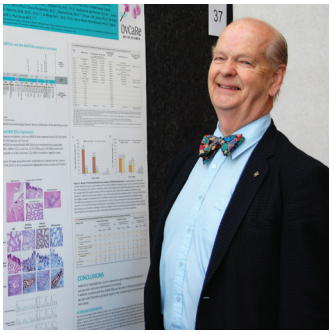
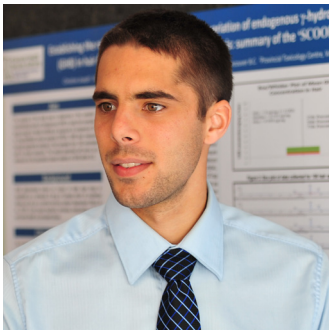
PATHOLOGY
laboratory medicine
UNIVERSITY OF BRITISH COLUMBIA





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THE UNIVERSITY OF BRITISH COLUMBIA

Abstract Book
Posters & Oral Presentations

PATHOLOGY DAY 2014

Acknowledgement

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2014 edition.

Gayla Johnson and Adeline Chan have been instrumental in handling the administrative and practical details of Pathology Day. Debbie Bertanjoli designed the website and managed the website tools in addition to preparing the abstract book.

We also wish to express our gratitude to faculty members who contributed their time and expertise to reviewing abstracts, moderating the oral sessions, and judging the oral and poster presentations. This year these include: Mike Allard, Kevin Bennewith, Cedric Carter, Hèléne Côté, Doug Filipenko, Diana Ionescu, Maria Issa, Karuna Karunakaran, Corree Laule, Anna Lee, Nikita Makretsov, Hamid Masoudi, Deborah McFadden, Vicky Monsalve, Torsten Nielsen, Mike Nimmo, Avi Ostry, David Schaeffer, Michael Seidman, Brian Skinnider, Decheng Yang, and Colby Zaph.

Finally, sincere thanks to staff who kindly assisted with technical and administrative support throughout the day and our photographers: Aleya Abdulla, Debbie Bertanjoli, Helen Dyck, Gayla Johnson, Andrew Leung, Jenny Tai, JJ Sun, Dmitry Turbin, and Jennifer Xenakis.

We hope you enjoy Pathology Day 2014.

The Pathology Day Committee
Mike Allard, Corree Laule, Avi Ostry and Jacqueline Quandt

Jacqueline Quandt, BSc, PhD (UBC), Assistant Professor

Avi Ostry, Clinical Associate Professor, UBC

Corree Laule, BSc, MSc, PhD Researh Associate

A Message from the Head

Michael F. Allard, BSc, MD, FRCP(C)
Professor and Cardiovascular Pathologist, UBC
James Hogg Research Centre
Heart + Lung Institute
St. Paul's Hospital

Pathology Day is a critically significant event in the departmental calendar as it serves as a time to showcase the broad spectrum of scholarly activities performed by our trainees and, by extension, our faculty. This gathering also provides a perfect venue to recognize outstanding contributions by members of the department. Pathology Day serves another very important function as well. It is an opportunity to get together to socialize and learn more about one another as well as gain an appreciation and understanding for the breadth of scholarly activities that take place in our geographically dispersed department. As for last year, a few members of our department will present a brief overview of their work as a way to facilitate this appreciation and understanding. We are very fortunate to have two outstanding individuals participate in the program this year, highlighting academic excellence and continuing in the tradition of having world leaders in their disciplines speak at Pathology Day. Dr. Jiri Frohlich will give the James Hogg Lecture, while Dr. Randall Thompson (Department of Cardiology, Saint Luke's Cardiovascular Consultants, Kansas City, Missouri) is our Keynote Speaker. I wish to extend my sincere thanks and gratitude to members of the committee responsible for organizing the event, including Drs. Jacquie Quandt, Avi Ostry and Corree Laule, Gayla Johnson, and Adeline Chan, as well as all the other individuals whose efforts make the day a success.

Hoping you enjoy this year's Pathology Day!

Warmest regards,

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Keynote Lecture

HOW OLD IS ATHEROSCLEROSIS? THE HORUS STUDY OF MUMMIES ACROSS 4000 YEARS

RANDALL C. THOMPSON, MD
Consulting Cardiologist
Saint Luke's Hospital of Kansas City
Professor of Medicine
University of Missouri School of Medicine
Kansas City, MO

Short Biography:

Dr. Thompson is originally from Atlanta, Georgia and Greenville, South Carolina and has practiced medicine with Saint Luke's Cardiovascular Consultants since 1995. He earned his undergraduate college and medical school degrees from Emory University in Atlanta, Georgia and served a residency in Internal Medicine and a fellowship in Cardiology at Massachusetts General Hospital and Harvard Medical School in Boston. Before moving to Kansas City, Dr. Thompson was consultant in cardiovascular diseases at the Mayo Clinic in Rochester, MN and Jacksonville, FL. Dr. Thompson's specialties include clinical cardiology and the fields of nuclear cardiology and cardiac and vascular CT. He is a member of the Board of Directors of the American Society of Nuclear Cardiology. Dr. Thompson has received numerous honors including AOA Medical Honorary Society, Top Docs of Kansas City, and the Alan Forker Cardiovascular Teaching Award.

Guest Speakers

1

DANA DEVINE, BA, MA, PHD

Professor, Pathology and Laboratory Medicine, UBC; Vice President, Medical, Scientific & Research Affairs, Canadian Blood Services; Associate Member, Biochemistry and Molecular Biology, Medicine

2

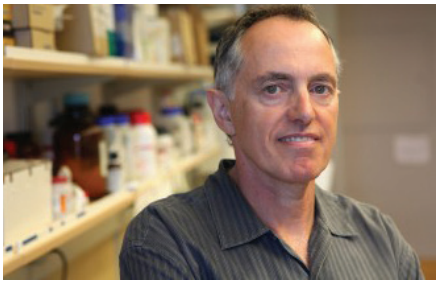
BRUCE VERCHERE, MSC, PHD

Professor, Pathology & Laboratory Medicine and Surgery; Head of the Diabetes Research Program at the Child & Family Research Institute (CFRI); Irving K Barber Chair in Diabetes Research

3

POUL SORENSEN, MD, PHD, FRCPC

Professor, Pathology & Laboratory Medicine; The Johal Chair in Childhood Cancer Research at UBC; Senior Scientist at the BCCA Research Centre Scientist at the CFRI



James Hogg Lecture

FAMILIAL HYPERCHOLESTEROLEMIA: FROM UNDERSTANDING THE PATHOGENESIS TO EFFECTIVE TREATMENT TO B.C. AND CANADIAN REGISTRY

JIRI FROHLICH, MD, FRCPC
Professor Emeritus, Pathology and Laboratory Medicine, University of British Columbia, Director of Clinical Trials, Healthy Heart Program Prevention Clinic

Short Biography:

Dr. Frohlich is a UBC Professor Emeritus of Pathology and Laboratory Medicine and Director of the Clinical Trials Division of St. Paul's Hospital Healthy Heart Program. Having obtained his medical degree from Karlova University, Prague in 1965, Dr. Frohlich relocated to Montreal in 1968 and worked as a research fellow and trained in internal medicine and clinical pathology at McGill University and UBC. Before assuming his current positions in 1995, Dr. Frohlich was the associate director of the department of pathology of the Shaughnessy/ University Hospital, in Vancouver, British Columbia.

Over the last 30 years, his service activities centered on establishing the first B.C. Lipid Clinic (1980) and being instrumental in the development of lipid clinic outreach programs throughout B.C. His research has focused on the biochemistry of lipoprotein metabolism, the pathology of atherosclerosis, and the development of new markers for the prediction of atherosclerosis. In addition to his service, research and teaching duties Dr. Frohlich also reviews numerous grants and publications and serves on the Editorial Board of scientific journals in the field of clinical biochemistry. Dr. Frohlich is a prolific author (co-author) of over 250 publications in journals such as Circulation, Laboratory Investigation, Nature, Atherosclerosis, Thrombosis and Vascular Biology, American Journal of Cardiology and Canadian Journal of Cardiology, among others dealing mostly with HDL metabolism, LCAT, clinical trials and guidelines.

Dr. Frohlich is a member of the Canadian Cardiovascular Society Dyslipidemia Working Group and the Canadian Cardiovascular Risk Assessment Working Group. He has also served on the British Columbia Heart Foundation Committee for the chair in cardiology. Dr. Frohlich is the president of the Healthy Heart Society of British Columbia (HHS) a non-profit organization which facilitates improvements in cardiovascular prevention practices in B.C. communities and collaborates with BCMA and Ministry of Health to improve management of chronic diseases. HHS received the BCMA "Excellence in Health Promotion Award" for these activities in 2005. In 2006 Dr. Frohlich received the UBC Department of Pathology and Laboratory Medicine's "Excellence in Service" Award.

CONFERENCE OUTLINE

Paetzold Health Education Centre, 1st Floor, JPPN, VGH

7:55am	Opening remarks – **Dr. Michael Allard**
8:00am	**JAMES HOGG LECTURE: Dr. Jiri Frohlich** - "Familial hypercholesterolemia: from understanding the pathogenesis to effective treatment to B.C. and Canadian registry" *(Lecture Theatre)*
9:00am	**GUEST SPEAKER: Dr. Bruce Verchere** - "Amyloid and inflammation in diabetes" *(Lecture Theatre)*

Graduate Student Oral Session *(Lecture Theatre)*

Abstract 9:30am-10:30am

#17. Hani Bagheri : Genomics of early pregnancy loss.

#18. Jay Gunawardana: Frequent and somatic mutations of PTPN1 in primary mediastinal B cell lymphoma and Hodgkin lymphoma.

#19. Charles Soong: Development of a next-generation sequencing-based platform to study DNA double-strand break repair mechanisms.

#20. Christa Klein-Bosgoed: Pathogen reduction technology impacts platelet mRNA: Reduction in GPIIIa mRNA amount does not affect protein expression.

Resident Oral Session *(Multipurpose Room)*

Abstract 9:30am-10:30am

#1. Nick Sunderland: MYC expression by immunohistochemistry predicts survival in mantle cell lymphoma.

#2. Peyman Tavassoli: Identification and development of a new class of androgen receptor antagonists with potential therapeutic application in advanced prostate cancer.

#3. Ian Garber: A novel deletion in the iron-response element of L-ferritin, causing hyperferritinemia cataract syndrome

#4. Ramesh Saeedi: Effects of liraglutide, a glucagon-like peptide-1 analogue, on metabolic abnormalities and body weight in HIV (+)ve patients with or without type 2 diabetes.

#5. Sophia Wong: Identification and quantification of insulin analogues by immunocapture coupled with liquid chromatography-tandem mass spectrometry

10:30am - 10:45 am	**Break (Atrium)**
10:45am - 11:05am	**GUEST SPEAKER: Dr. Dana Devine** - "Canada's Blood Supply: The Journey Toward Blood Safety" *(Lecture Theatre)*

Graduate Student Oral Session *(Lecture Theatre)*

Abstract 11:20am - 12:05pm

#21. Emma Conway: Characterization of macrophage populations in the lungs of healthy individuals and cancer patients.

#22. Alistair Chenery: Overlapping mucosal immunity: regulation of allergic lung inflammation by intestinal whipworms.

#23. Anthony Hsieh: Measuring the effects of chronic HIV infection and other clinical modulators on cellular aging in senescent cytotoxic T cells..

Resident Oral Session *(Multipurpose Room)*

Abstract 11:20am - 12:15pm

#6. Natalie Blaszczyk: Comparison of one stage clotting and two stage chromogenic factor VIII assay results and correlation of discrepant results with specific patient bleeding histories.

#7. Eman Khan: Audit of trauma exanguination protocol for management of trauma patients with massive bleeding.

#8. Habib Razavi: Linking testing-to-clinical goals - A risk stratified process re-design scheme towards improving manual blood film review (MBFR) services in a large tertiary hospital laboratory.

#9. Audi Setiadi: The significance of peripheral blood minimal residual disease to predict early disease response in patients with B-cell acute lymphoblastic leukemia.

#10. Ayesha Vawda: Cytogenetics is non-contributory in cases of cytopenia without morphological dysplasia.

12:30pm - 2:10pm	**Lunch and poster session (UBC Medical Student & Alumni Centre, 2750 Heather Street)**
2:30pm - 2:50pm	**GUEST SPEAKER: Dr. Poul Sorensen** - "Selective mRNA translation and the tumor cell stress response" *(Lecture Theatre)*

Graduate Student Oral Session *(Lecture Theatre)*

Abstract	3:00pm - 4:00pm
#24.	Kyle Burrows: The transcriptional repressor Hic1 modulates T cell fate and function.
#25.	Jesse Olson: Developmental exposure to maternal folic acid and vitamin B12 imbalance programs pancreatic islet mafa expression and glucose tolerance in female offspring mice.
#26.	Melissa Glier: The role of cystathionine-beta-synthase in obesity-related cardiac lipotoxicity.
#27.	Jaques Courtade: Impaired processing of human pro-islet amyloid polypeptide (proIAPP) promotes early islet graft failure.
#28.	Yuda Shih: Oligodendroglial protein tyrosine phosphatase alpha signaling is altered by wnt and promotes myelination in glial-neuron co-cultures.
#29.	Sophie Stukas: Intravenously injected human apolipoprotein A-I rapidly enters the central nervous system via the choroid plexus in mice.

Resident Oral Session *(Multipurpose Room)*

Abstract	3:03pm - 4:15pm
#11.	Michael Payne: Review of 16S and ITS direct sequencing results for clinical specimens submitted to a reference laboratory.
#12.	Lien Hoang: Degenerative changes in uterine leiomyomas during pregnancy and puerperium.
#13	Susanna Zachara: The accuracy of subclassifying poorly differentiated non-small cell lung carcinoma biopsies with commonly used lung carcinoma markers.
#14.	Veronica Hirsch-Reinshagen: Genomic and histological subclassification of adult medulloblastoma a study of patients treated at the Vancouver General Hospital.
#15.	Peter Schutz: Brain biopsy in pediatric inflammatory encephalopathy.
#16.	Max Signaevski: Cerebral hyaline astrocytic inclusions in treatment-resistant epilepsy and global developmental delay.

4:30pm - 5:30pm	**KEYNOTE LECTURE: Dr. Randall Thompson** - "How old is atherosclerosis? The Horus Study of Mummies Across 4000 years" *(Lecture Theatre)*
5:45pm	**Cocktails & Canape's (UBC Medical Student & Alumni Centre, 2750 Heather Street)**
6:30pm	**Awards (UBC Medical Student & Alumni Centre, 2750 Heather Street)** **EVENING EVENT CLOSES AT 8:30 PM**

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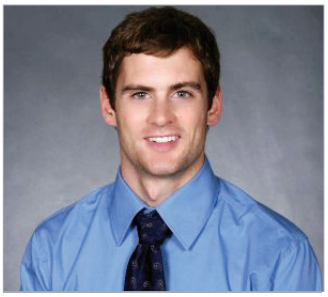
POSTER PRESENTATIONS

ABSTRACT # 1

NICHOLAS SUNDERLAND

Supervisor: Dr. Graham Slack
Session: Clinical Sciences

"MYC expression by immunohistochemistry predicts survival in mantle cell lymphoma"



AUTHORS:
NE Sunderland,[1] GW Slack,[1,2] RD Gascoyne[1,2]

AFFILIATIONS:
UBC Department of Pathology and Lab Medicine, [2]; BC Cancer Agency

Background/objectives:
Mantle cell lymphoma (MCL) is a B-cell lymphoma with a median survival of 3-5 years but some patients experience a more aggressive clinical course with rapid progression and shorter survival. The ability to detect these patients at the time of diagnosis may have clinical utility, however, only a few markers of aggressive behavior have been identified. MYC protein expression by immunohistochemistry (IHC) was recently identified as a prognostic marker in diffuse large B-cell lymphoma but its significance in MCL is not well known. The purpose of this study was to determine the clinical significance of MYC protein expression by IHC in MCL.

Methods:
176 cases of formalin-fixed paraffin-embedded MCL in a tissue microarray were independently evaluated by two pathologists for expression of MYC by IHC. MYC-positivity (MYC+) was defined as >20% lymphoma cells with nuclear staining. MYC expression was correlated with overall (OS) and progress-free survival (PFS) and the mantle cell lymphoma International Prognostic Index (MIPI). Clinical data were available for all patients.

Results:
The patient population consisted of 121 males and 55 females ranging from 22-91 years in age (median 68.5 years). MYC IHC showed a concordance of 97.2% between observers. 21 cases (11.9%) were MYC+. Univariate analysis using the Kaplan-Meier method and log-rank test showed MYC+ MCL, compared with MYC- MCL, was associated with shorter OS (median: 0.7 vs. 3.0 years, 5-year: 0.0% vs. 26.5%, $p<0.001$) and PFS (median: 0.3 vs. 1.2 years, 5-year: 0.0% vs. 11.0%, $p<0.001$). Cox regression multivariate analysis showed MYC+ was associated with shorter OS ($p<0.001$) and PFS ($p<0.001$) independent of MIPI.

Conclusions:
MYC IHC in MCL is a robust assay with high concordance between observers. MYC+ MCL occurs in 11.9% of cases and is an independent predictor of shorter OS and PFS. Additional studies comparing MYC IHC with other pathologic markers of aggressive behavior in MCL (ex. pleomorphic/blastoidcytology and proliferation rate) are ongoing.

PEYMAN TAVASSOLI

Supervisor: Dr. Paul Rennie
Session: Clinical Sciences

"Identification and development of a new class of androgen receptor antagonists with potential therapeutic application in advanced prostate cancer"

AUTHORS:
Peyman Tavassoli,[1] Peter Axerio-Cilies,[1] Nathan A. Lack,[1,2] Eric Leblanc,[1] Huifang Li,[1] Mohamed DH Hassona,[1] Natalia Kanaan,[1] Kate Frewin,[1] Kriti Singh,[1] Hans Adomat,[1] Helge Prinz,[3] Frank Q. Han,[1] Ka Hong Chan,[1] Emma Tomlinson Guns,[1] Paul S. Rennie,[1] Artem Cherkasov[1]

AFFILIATIONS:
[1]Vancouver Prostate Centre, University of British Columbia, Vancouver, Canada; [2]School of Medicine, Koc University, Istanbul, Turkey; [3]Institute of Pharmaceutical and Medicinal Chemistry, Westfälische Wilhelms-Universität, Hittorfstrasse, Münster, Germany

Prostate cancer, an androgen dependent cancer, is the most common non-skin cancer and one of the leading causes of cancer related death in men. It is often curable in early stages via surgery or radiotherapy. However, locally advanced, metastatic or relapsed prostate cancer is difficult to control. These patients are subject to androgen withdrawal therapies that are designed to either target androgen production or their binding to androgen receptor (AR). Although initially successful, cancer progress after a short period and become castration resistant prostate cancer (CRPC) with an overall survival of about 2 years. There is significant evidence that even at the CRPC state, AR often has a main role in tumor growth. In over 80% of locally advanced CRPC, high levels of nuclear AR have been observed and the amount of nuclear AR present in bone metastasis is often higher than in primary tumors. Therefore, targeting AR would be a practical therapeutic option in patients with CRPC.

Currently, there are many potent anti-AR drugs that all work through the same mechanism. They all compete with androgens for androgen binding site on AR. This site is a shallow pocket and not an appropriate therapeutic target. Furthermore, it is very prone to development of drug resistant. Recent studies have identified novel site on the AR called binding function 3 (BF3) that is involved in AR transcriptional activity. We have implemented a large scale virtual and experimental screening for compounds bound to BF3 and inhibits AR activity. 213 compounds are selected and are screened for inhibition of AR activity using cell based screening eGFP assay. In this assay, the expression of eGFP is under the control of an androgen responsive promoter and can quantify AR transcriptional activity. After removing compounds that exhibit non-specific cellular toxicity, 17 compounds are found to decrease AR activity more than 55%, some of them even more efficiently than bicalutamide, a conventional AR antagonist. Further analysis shows a lead compound, VPC-3033 (10-benzylidene-10H-anthracen-9), that is able to effectively inhibit AR transcriptional activity and cause profound AR degradation. Using xenograft prostate cancer model, VPC-3033 shows significant tumor growth suppression. Moreover, VPC-3033 demonstrates strong effect against prostate cancer cells that have already developed resistance to Enzalutamide (MDV3100), a new generation of anti-AR drug.

These results provide a foundation for the development of a new class of AR antagonists that can inhibit tumor growth and potentially control tumor burden in patients with advanced prostate cancer.

ABSTRACT # 3

IAN GARBER

Supervisor: Dr. Morris Pudek
Session: Clinical Sciences

"A novel deletion in the iron-response element of L-ferritin, causing hyperferritinemia cataract syndrome"

AUTHORS:
Ian Garber[1] and Morris Pudek[2]

AFFILIATIONS:
[1]Medical Biochemistry Resident, Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Division of Chemistry, Department of Pathology and Laboratory Medicine, Vancouver General Hospital

Background/objectives:
Hyperferritinemia cataract syndrome (HCS) is a rare genetic condition that results in very high ferritin levels without iron overload. It is an autosomal dominant condition caused by mutations in the iron-response element of L-ferritin (FTL). These mutations in the 5' untranslated region of FTL interfere with the binding of ferritin mRNA to iron regulatory protein, leading to translational derepression of ferritin synthesis and constitutively high production of ferritin protein, uncoupled from intracellular iron levels. Excess ferritin may precipitate in the lens of the eye, leading to bilateral early-onset cataracts, the only significant clinical consequence of this condition. It is important to recognize these patients - historically, misdiagnosis with hemochromatosis has led to iatrogenic anemia from therapeutic phlebotomy. The present case involved a 47-year-old woman with persistently elevated ferritin, discovered incidentally when screening for iron deficiency. This was associated with a transferrin saturation at the low end of the normal range. After ruling out acquired causes of hyperferritinemia as well as laboratory interferences, further questioning revealed that the patient had undergone bilateral cataract surgery in her early 20s. This information was sufficient for clinical diagnosis of HCS, but with the patient's permission, we decided to go one step further and obtain molecular confirmation by searching for FTL mutations.

Methods:
DNA was extracted from patient peripheral leukocytes. PCR primers flanking the iron-response element of FTL were synthesized, and used to amplify the region of interest. Agarose gel electrophoresis confirmed a single band of the expected size. The PCR product was gel extracted and cloned into a pCR2.1-TOPO vector prior to sequencing. Individual clones were picked and sequenced on an Applied Biosystems 3730xl analyzer, using standard M13 primers.

Results:
Comparison to the reference sequence revealed a heterozygous 4-base deletion in the iron-response element of FTL. This mutation has not been previously reported. It encompasses the cytosine bulge, a portion of the iron response element stem-loop structure known to be crucial for binding to iron regulatory protein..

Conclusions:
We have discovered a new HCS-causing mutation in this patient who presented with incidental hyperferritinemia.

RAMESH SAEEDI

Supervisor: Dr. Marianne Harris
Session: Clinical Sciences

"Effects of liraglutide, a glucagon-like peptide-1 analogue, on metabolic abnormalities and body weight in HIV (+)ve patients with or without type 2 diabetes"

AUTHORS:
Ramesh Saeedi[1], Parisa Dabirvaziri, Masoud Yousefi, Negar Fouladi, Silvia Guillemi[2], Greg Bondy[2], Marianne Harris[2]

AFFILIATIONS:
[1]Department of Pathology & Laboratory Medicine,UBC;
[2]BC Center for Excellence in HIV/AIDS, UBC

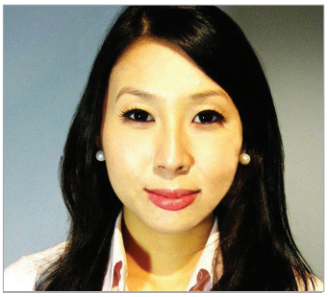
Background/objectives:
Once-daily liraglutide provides glycaemic control and lowers weight and systolic blood pressure (sBP) in obese patients with and without type 2 diabetes mellitus. Here we evaluate the effects of six-month liraglutide treatment on glycemic control, body weight, sBP, and lipid profile in obese HIV(+)ve subjects with and without diabetes.

Methods:
We performed a retrospective case control study in which medical records of HIV(+)ve and non-HIV patients with or without diabetes who were treated with liraglutide for 6 months were analyzed. Patients were separated into 4 groups: HIV(+)ve obese (N=30), HIV(+) with diabetes (N=32), obese (N=32), and diabetes (N=53). Changes in body weight, hemoglobin A1c (HgA1c) , sBP, plasma C-reactive protein (hsCRP) concentrations, fasting glucose (FBG) and serum lipids were investigated over 6 months of liraglutide treatment. The data were expressed as Mean ± Standard Error of Mean (M ± SEM). The data were analyzed using Repeated Measures Analysis Of Variance (RM-ANOVA) with Bonferroni's correction. P value less than 0.001 was considered to be statistically significant. All statistical calculations were performed with SPSS software package.

Results:
Body weights were reduced in all groups; these reductions were significant ($P<0.001$) in all except HIV(+)ve obese patients. HgA1c was significantly reduced in obese and diabetic non-HIV subjects ($P<0.001$). In HIV(+)ve obese patients, LDL-C and TC/HDL-C were reduced ($p<0.05$). Liraglutide treatment of HIV(+)ve patients with diabetes resulted in reductions in sBP, TC, and non-HDL-C ($P<0.05$). In obese patients, reductions in TC/HDL, TG, logTG/HDL, non-HDL-C and LDL-C were observed ($p<0.05$). In obese patients, FBG and TC were significantly reduced ($P<0.001$). In diabetic patients, liraglutide resulted in a reduction in sBP, non-HDL-C, FBG, and logTG/HDL-C ($p<0.05$).

Conclusions:
Liraglutide treatment of HIV(+)ve patients with and without diabetes over 6 months induced weight loss particularly in subjects with diabetes and improved certain metabolic risk factors.

ABSTRACT # 5



SOPHIA WONG

Supervisor: Daniel T. Holmes
Session: Clinical Sciences

"Identification and quantification of insulin analogues by immunocapture coupled with liquid chromatography-tandem mass spectrometry"

AUTHORS:
Sophia L Wong[1], Grace Van Der Gugten[2], Daniel T Holmes[1,2]

AFFILIATIONS:
[1]Division of Medical Biochemistry, Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Division of Clinical Chemistry, Department of Pathology and Laboratory Medicine, St. Paul's Hospital

Background/objectives:
Synthetic insulins, or insulin analogues, are routinely prescribed in type 1 and advanced type 2 diabetes mellitus. Detection and quantitation of synthetic insulins are useful in confirming accidental or intentional overdoses of insulin analogues, as well as in the workup of aberrant insulin results. In most laboratories, insulin concentration is determined by immunoassay. Insulin immunoassays exhibit variable cross-reactivities to synthetic insulins, are prone to interferences from insulin precursors/degradation products/autoantibodies, and fail to provide information on the type of analogue present. Given these limitations, we have developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the identification of 5 commonly prescribed pharmaceutical insulins.

Methods:
25 uL of 500 uU/mL bovine insulin (internal standard), and 5 uL of 5 g/L dextran sulfate + 0.5 M MgCl2, were added to 500 uL of patient serum or calibrator (insulin analogues in 20% acetonitrile) in Eppendorf LoBind tubes. The mixtures were incubated with 250 uL of paramagnetic beads coated with monoclonal mouse anti-insulin antibodies at room temperature for 1 hour. The beads were washed 3 times with 1 mL 0.01 M phosphate buffered saline (PBS), and the insulin analogues extracted with 2 x 75 uL 1% acetic acid into a bovine serum albumin (BSA)-treated 96-well plate. Chromatographic separation was achieved with an ACE 300 C18 column (5 x 2.1 mm, 5 um ID) with a run-time of 8.5 minutes. The samples were analyzed on an AB SCIEX QTRAP 5500 system in positive electrospray ionization (ESI) mode, with multiple reaction monitoring (MRM) transitions monitored for regular insulin (qualifiers m/z 1162/226, 1162/652; quantifier m/z 1162/345), lispro (qualifier 969/217; quantifier 1162/217), aspart (qualifiers 972/226, 1166/219; quantifier 972/136), detemir (qualifiers 987/454, 1184/357; quantifier 1184/454), and glargine (qualifiers 1011/1164, 1011/1179; quantifier 867/136). Calibration curves were constructed with linear regression using 1/x weighting.

Results:
The method demonstrated good linearity over a concentration range of 5-200 uU/mL, with R>0.997 for all insulin analogues. Analytical recoveries were between 90.3% and 113.4%. Approximate lower limit of detection (LLOD) and lower limit of quantification (LLOQ) were 3.5 uU/mL and 5 uU/mL, respectively. Within-run coefficients of variation (CVs) ranged from 3.2% to 14.8%. The utility of the method was shown in a series of case studies.

Conclusions:
We have developed a robust LC-MS/MS assay for the quantitation of 5 popular insulin analogues. It is valuable in the workup of insulin-related clinical and forensic cases, and has overcome some of the limitations exhibited by current commercial insulin assays.

NATALIA BLASZCZYK

Supervisor: Dr. Shannon Jackson
Session: Clinical Sciences

"Comparison of one stage clotting and two stage chromogenic factor VIII assay results and correlation of discrepant results with specific patient bleeding histories"

AUTHORS:
Natalie Blaszczyk, Shannon Jackson, Steven Wong, Nadejda Medvedev, Deborah Griswold, Cedric Carter

AFFILIATIONS:
[1]University of British Columbia, Department of Pathology and Laboratory Medicine

Background/objectives:
It is a known phenomena that a small proportion of patients with Hemophilia A demonstrate discrepant results between their factor VIII levels as measured by the one stage factor assay and their bleeding tendencies. Most of these patients fall into the mild Hemophilia A category, with occasional cases in the moderate category. It has been shown that their bleeding phenotype may be better reflected in the two stage chromogenic assay, which can sometimes yield significantly lower factor levels than the standard one stage assay.

Methods:
We conducted a small scale study at our institution comparing factor levels as measured by a one stage factor VIII assay from Instrument Laboratories versus a biophen chromogenic assay from Anira. We then examine the bleeding tendencies/clinical histories of the patients with discrepant assay results.

Results:
A total of 22 patients were examine, 6 had severe factor deficiency, 4 had moderate and 12 had mild. 3 of the patients were on factor replacement therapy (2 severe and 1 mild). There were no discrepancies between the two assays for the patients on factor replacement therapy. 8 patients had numerically discrepant results between the one and two stage assays, 7 results were lower when measured with the two stage chromogenic and 1 result was higher. Of the 4 patients with moderate Factor VIII deficiency, 3 had discrepant results, one of whom had an increased bleeding tendency than could potentially be expected by his factor levels based on the one stage assay. The clinical bleeding presentation in this patient was more in line with the two stage chromogenic assay levels. Of the 12 mild cases, 4 had discrepant results. 3 of these 4 patients had increased bleeding tendencies than could potentially be expected based on the one stage factor VIII assay, again more in line with the two stage chromogenic assay results. One of these patients had a low level inhibitor, previously documented. Of the 6 severe cases 1 had a discrepant numerical result, however, due to the fact that such patients have severe clinical symptoms the assay result would not impact the treatment he receives. Of note among the patients with discrepant numerical results were two related males with mild Hemophilia A. Both had significantly lower levels of factor VIII as measured using the two stage chromogenic assay than the one stage assay based on which their disease is currently classified. However, most interestingly, only one of the patients, the younger one, had evidently higher bleeding tendencies. This observation suggests other modifying factors may play a role in the clinical presentation of this particular familial mutation in the Factor VIII gene.

Conclusions:
In conclusion, the results suggest that perhaps some patients could benefit from more aggressive treatment than is currently recommended for them based on their present classification into moderate and mild categories, which has been derived from the standard one stage Factor VIII assay results.

ABSTRACT # 7

EMAN KHAN

Supervisor: Dr. Kristine Roland and Dr. Katherine Chipperfield
Session: Clinical Sciences

"Audit of trauma exanguination protocol for management of trauma patients with massive bleeding"

AUTHORS:
Eman Khan[1], Katherine Chipperfield[1], Kristine Roland[1]

AFFILIATIONS:
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Background/objectives:
Severely injured patients can develop acute coagulopathy triggered by the trauma itself and leading to massive hemorrhage. Retrospective studies have demonstrated improved survival in trauma patients who receive early Frozen Plasma (FP) in a 1:1 ratio to Red Blood Cells (RBC). Based on this literature a Trauma Exsanguination Protocol (TEP) was implemented at our hospital in August 2011 in which a 1:1 ratio of FP:RBC is issued by the Transfusion Medicine Service (TMS) in validated transport coolers. Shortly after, the massive transfusion consensus panel 2011concluded that supporting evidence of such strategy is not enough to recommend this strategy as the standard of care, an internal audit of our TEP was conducted to ensure compliance with the protocol, efficacy of the intervention, and avoidance of unnecessary wastage of blood products.

Methods:
This was a retrospective review of TEP forms, patient charts and the hospital laboratory information system for all TEP activations at Vancouver General Hospital between August 2011 and August 2013.

Results:
There were 41 TEP activations during the study period. The most common type of injury requiring TEP activation was motor vehicle accident 12/41(29%). All predetermined criteria for activating the TEP was met in 47% of cases. Patients were transfused on average 16 units of RBC and 6 units of FP. The average ratio of FP:RBC transfused in 24 hours was 1:3. The average Hemoglobin (Hb), platelets (plt) and INR upon arrival was 116 g/L, 194 x109/L and1.4 respectively; While the average Hb, plt and INR at 24 hours was 109 g/L, 100 x109/L and 1.2. The average turn around time from TEP activation to the release of the first cooler of blood was 15 minutes. The average unused products were 8.5 RBC and 5 FP per patient; most of these units could be returned into TMS inventory.

Conclusions:
Issuing blood in a 1:1 ratio does not necessarily ensure that patients are transfused in a 1:1 ratio; contributing factors will need to be explored. The predetermined criteria for activating the TEP at our hospital are often not met and therefore should be reviewed. Although AB plasma usage appears to be increased after TEP implementation, the discard rate did not go up. This is also true for the RBC and FP as a significant number of units issued were ultimately not transfused but wastage rates can remain low if attention is made to proper storage and transport practices.

HABIB MOSHREF RAZAVI

Supervisor: Dr. David Pi
Session: Clinical Sciences

"Linking testing-to-clinical goals - A risk stratified process re-design scheme towards improving manual blood film review (MBFR) services in a large tertiary hospital laboratory"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
A hematopathologist's review and comment (HPCOM) on blood film can be of critical importance in the initial diagnostic work up of a hematological disease. Recent feedback from the clinical users verified that HPCOM information is generally not available for timely clinical decision making and patient management. This paper presents the development and implementation of a process redesign project, aiming to improve the turn-around-time (TAT) of critical HPCOM results to meet clinical user's expectation in a high volume large tertiary hospital laboratory.

Methods:
The key components of the logic model include: (1) A risk stratification scheme to separate blood film morphology abnormalities - (i) minor/low diagnostic specificity entities, as Disease Associated Abnormalities (DAA), or (ii) major/clinical significant entities, as Disease Defining Abnormalities (DDA). For DAA, the use of an intelligent data manager (MiddlewareTM), permits the development of customized alert codes/decision functions for some numerical abnormalities, as a diagnostic aid, based on pre-defined logics in the transactions of laboratory information to clinical users. (2) For DDA, the implementation goal is to upkeep/advance the diagnostic acuity of the front-line technologists. An Inter-professional Learning (IPL) model is designed to foster a codependent learning opportunity between the technologists and pathologists. The combined strategies aim to cope with the technologists' workload in managing DAA cases, while enhancing their core competence in recognizing critical morphological abnormalities and refer them to a pathologist for review on a priority basis. Anonymous LIS data at Vancouver General Hospital before and after program implementation were extracted and analyzed for performance evaluation.

Results:
Despite an increase in CBC test volume of 6.5% (23,843 vs. 25,380 respectively) in the study period (January - 2013 and 2014), before and after program implementation, the rate of MBFR by technologists remained constant (4,337 vs. 4,666; 18.2% vs. 18.4% respectively). The HPCOM cases declined substantially (1,024 vs. 839; 4.3% vs. 3.3% of total respectively). A significant reduction in TAT for HPCOM was evident: (1) Median TAT (14.6 vs. 6.8 hours respectively; $p<0.0001$), (2) TAT cut-off ≤ 6 hours (27.3% vs. 46.1% respectively; $p<0.0001$.

Conclusions:
The standard MBFR process relies on a sequential transactional pathway, resulting in significant time delay in the final release of HPCOM information to clinical users. This is a proof-of-concept model which demonstrated that risk stratification/prioritization and knowledge sharing in a case-based learning environment are effective strategies and can improve the clinical goals of MBFR services.

ABSTRACT # 9

AUDI SETIADI

Supervisor: Dr. Suzanne Vercauteren
Session: Clinical Sciences

"The significance of peripheral blood minimal residual disease to predict early disease response in patients with B-cell acute lymphoblastic leukemia"

AUTHORS:
Audi Setiadi[3], Daniel Owen[3], Angela Tsang[1], Ruth Milner[1,2], Suzanne Vercauteren[1,2,3]

AFFILIATIONS:
[1]BC Children's Hospital; [2]Child & Family Research Institute; [3]Department of Pathology and Laboratory Medicine, Hematopathology

Background/objectives:
In patients with acute lymphoblastic leukemia (ALL), detection of minimal residual disease (MRD) in the bone marrow post-induction therapy is one of the most important prognostic factors and has now become the standard of care in these patients. Some studies have investigated the use of MRD testing at earlier time points (day 8 or 15) in peripheral blood to measure early response to therapy, but the optimal time point and level of MRD has not been well established. We assessed whether MRD detection by flow cytometry in peripheral blood (PB) at earlier time points during the treatment (day 8 and 15) is predictive of MRD level in the bone marrow (BM) at the end of induction (day 29). We also sought to determine the optimal cut off level of PB MRD to predict early response with the goal to replace bone marrow assessment at the earlier time points.

Methods:
In this retrospective study, we performed MRD flow cytometry analysis in samples from 77 patients of new or relapsed B-ALL received at the BC Children's Hospital between April 2010 and November 2013. All patients have received induction treatment using the established protocol at the BC Children's Hospital. All specimens had flow cytometry testing done in BM at day 0 (diagnosis) and day 29 of induction therapy using the following panel: Tube 1: CD45-PC5, CD19-PC7, CD20-PE, CD10-ECD and CD38-FITC; Tube 2: CD45-PC5, CD19-PC7, CD13+CD33-PE, CD10-ECD and CD34-FITC. MRD levels in peripheral blood were determined at day 8 and 15 using a one-tube panel: CD45-PC5, CD19-PC7, CD20-ECD, CD10-PE and CD34-FITC.

Results:
Of the 77 patients (39 males, 38 females), 76 had BM MRD testing at day 29, 74 and 56 patients had PB MRD testing at day 8 and day 15, respectively. Of the 76 patients, 61 patients (80.2%) were BM MRD negative at day 29. All patients with PB MRD <0.01% at day 8 (n=10) and day 15 (n=23) were BM MRD negative at day 29 (sensitivity 100%). Fifteen patients (19.7%) were MRD positive (≥0.01%) in the BM at day 29. Out of these 15 patients, 13 and 10 had day 8 and day 15 PB MRD done, respectively. All 13 patients (100%) had PB MRD ≥=0.01%, 11/13 (84.6%) had PB MRD ≥ 0.1% and 6/13 (46.2%) had PB MRD ≥1% at day 8. At day 15, 10/10 (100%) had PB MRD ≥0.01%, 8/10 (80.0%) had PB MRD ≥0.1% and 3/10 (30.0%) had PB MRD ≥1%. BM MRD positivity was detected in 20.0%, 23.4%, 28.6% of patients with day 8 PB MRD of ≥0.01%, ≥0.1%, ≥1%, and 31.3%, 50.0%, 75.0% of patients with day 15 PB MRD of ≥0.01%, ≥0.1%, ≥1%, respectively. PB MRD of 1% at day 15 has the highest specificity (97.8%) and positive predictive value (75%), but the lowest sensitivity (30%) and negative predictive value (86.3%). Statistical analysis is pending.

Conclusions:
MRD can be detected in peripheral blood at day 8 and 15 using a single tube flow cytometry method. MRD levels with a cut-off of 0.01% at day 8 are not very predictive of positive MRD after induction chemotherapy. At day 15, MRD level of 1% is the most predictive of end of induction MRD levels. Peripheral blood MRD testing at these earlier time points could offer a less invasive alternative to bone marrow procedures.

AYESHA VAWDA

Supervisor: Dr. Hélène Bruyère
Session: Clinical Sciences

"Cytogenetics is non-contributory in cases of cytopenia without morphological dysplasia"

AUTHORS:
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AFFILIATIONS:
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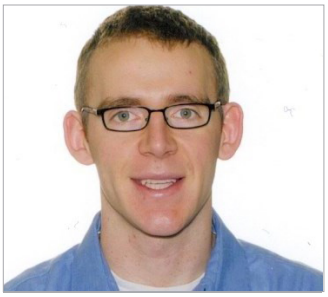
Background/objectives:
Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic disorders that present with cytopenias and morphological features of dysplasia. Approximately 50% of MDS patients have clonal cytogenetic abnormalities, which are important for diagnosis and prognosis. We investigated the diagnostic utility of cytogenetics in cases of uni-, bi-, and pancytopenia and clinical suspicion of MDS without clear morphological evidence of dysplasia on bone marrow (BM) examination.

Methods:
Patient records from Vancouver General Hospital Cytogenetic Department database from 2011 and 2012 were reviewed. One hundred patients with a BM biopsy performed to rule out MDS in the context of uni-, bi-, or pancytopenia with no clear morphological evidence of dysplasia were identified. Information recorded was: gender, age, BM biopsy indication, final BM diagnosis, differential diagnosis, peripheral blood counts (hemoglobin, white blood cell, neutrophil, and platelet count), BM blast %, whether cytogenetics was performed/reactivated, and karyotype.

Results:
There were 61 males and 39 females included in the analysis. Patient age ranged from 20 to 89 years with median age of 57 years and mean age of 56 years. Fifty patients had unicytopenia, 16 had bicytopenia and 31 had pancytopenia. Three patients had a history of cytopenia not documented on date of BM biopsy. Cytogenetics was performed in 40 cases; 3 failed to provide metaphases. Of the 37 karyotypic analyses done, abnormalities were identified in only 2 cases: a 5q deletion in 2 out of 75 metaphases from a mildly anemic patient; a 9;19 translocation in all 20 metaphases suspected to be constitutional in a pancytopenic patient. The laboratory cancelled cytogenetics in 60 cases. Six cases were reactivated upon clinician requests, all of which had a normal karyotype.

Conclusions:
Our preliminary results suggest that despite the presence of cytopenia(s), cytogenetics is non-contributory to patient diagnosis and management in the absence of clear morphological evidence of dysplasia. As a next step, we plan to validate this data in a larger cohort and design laboratory guidelines for cytogenetic testing in suspected MDS. This would have a positive impact on workload, specimen turn-around time and improve cost-efficiency in a resource-constrained public health care system.

ABSTRACT # 11



MICHAEL PAYNE

Supervisor: Dr. Linda Hoang
Session: Clinical Sciences

"Review of 16S and ITS direct sequencing results for clinical specimens submitted to a reference laboratory"

AUTHORS:
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AFFILIATIONS:
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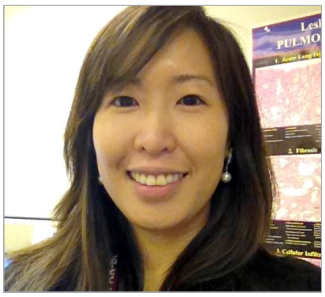
Background/objectives:
To evaluate the performance of 16S and internal transcribed spacer region (ITS) amplification and sequencing of rDNA from clinical specimens, for the respective detection and identification of bacterial and fungal pathogens.

Methods:
Direct rDNA amplification of 16s and ITS targets from clinical samples were experimentally performed over a 4 year period (01/2009-06/2013), and reviewed. All specimens were from sterile sites, and submitted to a reference laboratory for evaluation.

Results:
A total of 277 16S tests were performed, with 64 (23%) positive. Specimens were composed of: 148 fresh tissues, 122 sterile fluids, and 7 paraffin embedded samples. Fresh tissues had amplified product in 33/148 (22%), compared to 28/122 (23%) for sterile fluids. Identification of an isolate was significantly more likely in microscopy positive 16S samples 14/21 (67%), compared to 35/175 (20%) of microscopy negative samples (p= <0.0001). A total of 110 ITS tests were performed, with 14 (13%) positive. Specimens were composed of: 43 fresh tissues, 34 sterile fluids and 33 paraffin embedded tissues. Fresh tissue specimens had amplified product in 6/43 (14%), and sterile fluids yielded 3/34 (9%). Of the 33 paraffin embedded tissues 5 had amplified product (15%). The yield of microscopy positive ITS samples, 9/44 (21%), was higher than microscopy negative samples 3/50 (6%), but failed to reach significance (p=0.06). The rate of successful amplification and identification for bacterial 16S sequencing was significantly higher than fungal ITS sequencing (p=0.02).

Conclusions:
A 4 year evaluation of 16S and ITS sequencing performed by our reference center was reviewed. Our results show a significantly higher yield with 16S in sterile samples where microscopy evaluation was positive, when compared to microscopy negative samples. Direct ITS sequencing tended towards a higher yield from fresh tissues with a positive microscopic examination. Given these findings, 16S and ITS are a valuable option for culture negative specimens from sterile sites, particularly in the setting of positive microscopy findings. With negative microscopy, the poor sensitivity of 16S and ITS in detecting and identifying an infectious agent needs to be considered.



LIEN HOANG

Supervisor:
Session: Clinical Sciences

"Degenerative changes in uterine leiomyomas during pregnancy and puerperium"

AUTHORS:
Lien N Hoang[1], Eric J Yang[2], Bradley J Quade[2], Marisa R Nucci[2]

AFFILIATIONS:
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Background/objectives:
Uterine leiomyomas are by far the most common neoplasms of the female genital tract. Their incidence increases steadily over the reproductive age period. Thus, as more women trend towards childbearing at a later age, the number of leiomyomas encountered during pregnancy rises correspondingly.

Aside from red degeneration, the spectrum of degenerative changes that can occur in uterine leiomyomas during pregnancy has not been well documented. Such degenerative changes can be disconcerting to many pathologists, and make the distinction between a benign and malignant smooth muscle neoplasm incredibly challenging.

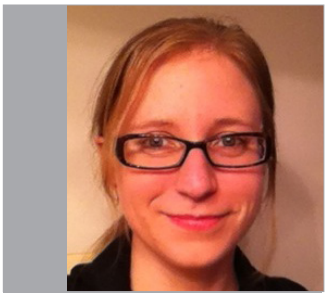
Methods:
We evaluated the gross and histopathologic features of a consecutive series of leiomyomas removed during pregnancy and puerperium. Cases were retrieved from a single institution between the years 2005-2010. We also reviewed each patient's electronic chart (3 to 9 year follow-up period) to determine if any had subsequently developed a borderline or malignant smooth muscle neoplasm.

Results:
One hundred and fourteen leiomyomas from 109 patients were evaluated. The average age of patients was 36.8 ± 5.0 years (range: 24 to 55 years) and the average size of the leiomyomas removed was 3.3 ± 2.1 cm. At least one type of degenerative change was seen in 71/114 (62.3%) of cases. Necrosis was present in 56/114 (49.1%) leiomyomas and 23 had necrosis surrounded by a hypercellular rim. Hemorrhage was present in 15/114 (13.2%). The prototypical changes of red degeneration was seen in only 6/114 (5.2%). Nuclear atypia was present in 36/114 cases (31.6%). No severe or diffuse atypia was identified. Mitotic activity was ≤4/10 high power fields, except in 3 cases where the mitotic rate was 7, 8, and 10 mitoses per 10 high power fields. Stromal hyalinization was evident in 58/114 (50.9%), edema 14/114 (12.3%) and calcification in 10/114 (16.7%). No intravascular thrombi or fibrinoid necrosis of the blood vessels were noted.

In the follow-up period, four patients had subsequent myomectomies or hysterectomies for recurrent leiomyomas. No patients had a subsequent diagnosis of a borderline or malignant smooth muscle neoplasm.

Conclusions:
A myriad of degenerative changes are seen in gestational leiomyomas, and caution should be taken to not mistake these changes for a malignant smooth muscle neoplasm.

ABSTRACT # 13



SUSANNA ZACHARA

Supervisor: Dr. Andrew Churg
Session: Clinical Sciences

"The accuracy of subclassifying poorly differentiated non-small cell lung carcinoma biopsies with commonly used lung carcinoma markers"

AUTHORS:
S. Zachara, T. Verdun, A. Churg

AFFILIATIONS:
[1]Department of Pathology & Laboratory Medicine, UBC

Background/objectives:
The recent development of targeted therapies has magnified the importance of differentiating between squamous cell carcinoma (SCC) and adenocarcinoma (AC) on biopsy specimens. The biopsy is a fractional representation of the whole tumour, and the small sample size often makes architectural interpretation difficult. The recent literature has applied immunohistochemical staining (IHC) to attempt to subclassify non-small cell lung cancer (NSCLC) into AC and SCC. The aim of this study was to assess the accuracy of IHC in subtyping poorly differentiated NSCLCs on biopsy when compared to their corresponding surgical resections.

Methods:
Twenty-four cases of NSCLC that could not be subclassified on hematoxylin and eosin (H&E) stained biopsy specimens alone and had subsequent resection specimens were identified. The resection specimens were considered the gold standard, and the tumour was classified based on the World Health Organization criteria. All biopsies and resection specimens were reviewed along with any IHC markers (generally TTF-1, Napsin, p63/p40, and CK5 or a subset of these markers) that were used to aid diagnosis.

Results:
According to biopsy specimen diagnoses, the 24 cases consisted of 9 SCCs, 8 ACs, and 7 NSCLC not otherwise specified (NOS). The resection specimens changed the count to 10 SCCs, 9 ACs, 4 large cell lung carcinomas (LCLC), and one adenosquamous carcinoma. 18 of the 24 cases (75%) had no change in diagnosis following primary tumour resection. Of the 6 biopsies that had an alternate final diagnosis, 4 were originally classified as NOS and subclassified to either AC (3 cases) or SCC (1 case) on the surgical specimen. One SCC was changed to adenosquamous carcinoma. One AC was changed to LCLC. The IHC staining patterns were 100% congruent between the biopsies and surgical resection specimens. The majority of diagnosis changes were due to architectural features that become more apparent on the larger resection specimens rather than any change in IHC staining. The one exception was a case with focal Napsin staining that was interpreted as consistent with AC, but discounted on the surgical resection specimen for a diagnosis of LCLC as the focal nature was much more evident.

Conclusions:
IHC staining using a combination of lung carcinoma markers allows accurate subclassification of poorly differentiated NSCLCs on small lung biopsies in most cases. Discrepancies in IHC staining between biopsies and resections are rare, and are due to interpretation difficulties rather than discordant staining. Surgical specimens allow for further subclassification mainly due to architectural features that are more apparent on whole resection.

VERONICA HIRSCH-REINSHAGEN

Supervisor:
Session: Clinical Sciences

"Genomic and histological subclassification of adult medulloblastoma a study of patients treated at the Vancouver General Hospital"

AUTHORS:
Veronica Hirsch-Reinshagen[1], Joanna Triscott[2], Sandra Dunn2, Brian Toyota[2], Christopher Dunham[1] and Stephen Yip[1]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, UBC, Canada; [2] Department of Pediatrics, UBC, Canada

Background/objectives:
Medulloblastoma is a small round blue cell tumor of the cerebellum that constitutes one of the most common malignant brain tumors of childhood. It can also present in adulthood, with up to 25% of all medulloblastomas occurring after 20 years of age. It is an aggressive, embryonal type of central nervous system tumor requiring aggressive therapeutic approaches with significant treatment-related morbidity. Recent advances at dissecting the molecular genetics underlying the disease have led to the discovery of at least four discrete molecular subgroups in pediatric patients: WNT (Wingless), SHH (Sonic hedgehog), Group 3, and Group 4. These subgroups exhibit highly distinct transcriptional and genetic profiles, patient demographics, and clinical behavior. It is expected that targeted treatment modalities and/or treatment protocols will be developed based on this molecular signature and incorporated into clinical practice in the near future. However, little is known about the clinical and molecular features of adult medulloblastomas, as less than 15% of the published data corresponds to patients over 16 years of age with one of the largest series composed of 29 adult cases. We therefore sought to evaluate the clinical, histological and molecular characteristics of adult medulloblastomas in adult patients diagnosed at Vancouver General Hospital over the last 16 years.

Methods:
Standard chart and histology review were used to evaluate clinical and histological characteristics. Nanostring nCounter technology was used to assess RNA expression profiles from archived FFPE tumor blocks. Then hierarchical clustering and PAM statistical methodology was employed to assign molecular subgroups.

Results:
Twenty-six patients with diagnosis of medulloblastoma were identified between 1998 and 2013, for three of which no histological material was available. Two additional patients were excluded from the study upon histology review. Histologically, 17 out of 21 cases were of the 'classical' subtype, while 4 were classified as 'desmoplastic/nodular'. By molecular subtyping using NanoString, most cases were classified to be in the SHH (X/Y). There was only one case that was WNT and one that was a Group 4. Interestingly, there were no Group 3 tumours identified. Only one case showed strong p53 staining. In addition, no cases showed loss of INI1 protein expression.

Conclusions:
In summary, this relatively large cohort of adult medulloblastomas conforms to previously described clinical, histological and molecular characteristics of this type of tumor. In addition, it provides a unique opportunity to further explore associated molecular changes that may be relevant in the prognosis and/or treatment of this aggressive disease.

Funding: BrainCare BC, Michael Cuccione Foundation and Hannahs Heros

ABSTRACT # 15

PETER SCHUTZ

Supervisor: Dr. Christopher Dunham
Session: Clinical Sciences

"Brain biopsy in pediatric inflammatory encephalopathy "

AUTHORS:
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AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine; [2]Division of Pediatric Neurology; [3]Division of Neurosurgery; University of British Columbia

Background/objectives:
In cases of pediatric encephalopathy of suspected inflammatory etiology the differential diagnosis chiefly involves vasculitis and encephalitis. In children, CNS vasculitis usually is lymphocytic, non-necrotizing and affects small-vessels (SVcPACNS). Differentiating vasculitis from encephalitis is crucial given significant differences in medical treatment. Unfortunately, histologic criteria for the diagnosis of SVcPACNS are somewhat vague. Our primary goal was to review the BCCH experience with cases of pediatric encephalopathy of suspected inflammatory etiology undergoing biopsy.

Methods:
Retrospective clinicopathologic review of cases of `acquired pediatric encephalopathy of suspected inflammatory etiology' was undertaken. All cases leading to brain biopsy at BCCH from 2000-2013 were included. Histologic review was initially directed by criteria set forth by Elbers et al. (2010) and Kennard et al. (1981).

Results:
17 biopsies were identified from 16 patients. 13/17 biopsies exhibited inflammation either characteristic of vasculitis (10/17; predominately SVcPACNS) or of encephalitis (3/17), while 4/17 biopsies displayed non-specific pathologic changes. The pattern of vascular inflammation seen in vasculitic and encephalitic cases was noted to overlap in some instances. The latter prompted us to propose revised histologic criteria for the diagnosis of pediatric SVcPACNS: 1) angiocentric predominant inflammation; 2) +/- mild diffuse microglial activation; 3) absence of or only rare microglial nodules; 4) absence of neuronophagia.

Conclusions:
Brain biopsy in the context of pediatric encephalopathy of suspected inflammatory etiology has a good diagnostic yield. Published histological criteria, however, have somewhat limited diagnostic utility. Revised criteria for the histologic diagnosis of pediatric SVcPACNS are suggested.

MAXIM SIGNAEVSKI

Supervisor: Dr. Christopher Dunham
Session: Clinical Sciences

"Cerebral hyaline astrocytic inclusions in treatment-resistant epilepsy and global developmental delay."

AUTHORS:
Maxim Signaevski[1], Chinnuwat Sanguansermsri[2], Christopher Dunham[1]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, Division of Neuropathology; [2]Department of Pediatrics, Division of Neurology

Background/objectives:
Cerebral hyaline astrocytic inclusions (HAI) have been observed in a subset of patients with epilepsy, brain structural anomalies, and developmental delay. These features suggest HAI may represent a unique clinicopathologic entity. We present a case of a 2.5-year-old male with epilepsy and global developmental delay. Chromosomal array detected a copy loss at 22q13.33 that resulted in a partial deletion of SHANK3 gene. This mutation caused variable dysmorphic features, global developmental delay/intellectual disability, absent to severely delayed speech, autism and epilepsy. The multiple interictal and ictal phase EEGs revealed that the seizure activity was arising from left frontal central region. MRI of the brain showed no abnormalities. The invasive video electrocorticography captured 6 clusters of epileptic spasms, all originating from left antero-lateral frontal lobe that was well rostral to the motor cortex.

Methods:
We utilized routine histology and immunohistochemistry to identify the inclusions and mapped their distribution in the resected portion of the cortex. We then superimposed this map onto the EEG and electrocorticographic data to evaluate for any correlation.

Results:
Histologic analysis revealed the presence of HAI in the posterio-medial portion of the resected cortex, which corresponded to the site of onset and generalization using the electrophysiology. We also found that HAI at the resection margin. Immunohistochemistry was largely non-contributory re: inclusion distribution.

Conclusions:
HAI is a rare but emerging entity that is associated with epilepsy. To our knowledge, the distribution of inclusions in HAI has never been mapped to electrophysiologic data in any reported case in the literature. Our work suggests that seizure onset and generalization in our case correlated with the distribution of inclusions.

This suggests that the inclusions may:
- play a role in epileptogenesis; or,
- be a biomarker of disease distribution.

Finally, the presence of the HAI at the resection margin may foreshadow future seizure activity in this patient.

HANI BAGHERI

Supervisor: Dr. Evica Rajcan-Separovic
Session: Basic Sciences

"Genomics of early pregnancy loss"

Background/objectives:
Miscarriage is the spontaneous termination of a clinically recognized pregnancy in utero before 20 weeks of gestation. It is estimated to occur in 10-15% of all pregnancies. The majority of miscarriages are caused by large-scale chromosome abnormalities, however in ~40% of cases, the chromosomes are normal and the reason for miscarriage remains unknown. The underlying cause for these cases was speculated to be due to submicroscopic DNA gains and losses known as copy number variations (CNVs) which can be detected by whole-genome array technology. In this study, our aim was to determine the genomic characteristics of CNVs detected in miscarriage, the function of selected genes from CNVs in miscarriage cells and presence of small pathogenic mutations in miscarriages as determined by exome sequencing.

Methods:
The genomic characteristics of CNVs were assessed by comparing the size and gene density/content of 24 rare (not seen in controls) and 132 common (present in controls) CNVs detected in a total of 101 reported euploid miscarriages using Wilcoxon rank-sum test. In addition, the gene density of common and rare CNVs was separately compared with 1000 equally-sized, randomly-distributed CNVs from the reference genome. The RNA and protein expression of 14 miscarriage genes was assessed by real-time qPCR and Western blotting in primary human trophoblast cell cultures from miscarriage placentae with CNVs (N=3) in comparison to cell cultures from elective abortions (N=4). We also carried out exome sequencing for 3 families with recurrent miscarriages. Bioinformatics analysis of genes integral to CNVs and affected by mutations as a pool was performed to identify enriched pathways using DAVID and WebGestalt web-tools.

Results:
Our results indicated a significantly (p=0.02) higher gene density in rare CNVs than common CNVs (64.9 vs 26 genes/Mbp) despite a comparable size of CNVs from these two groups (~0.15Mbp). RNA and protein expression results showed that 3/14 tested CNV genes had altered expression in miscarriage tissues. Two of these genes (TIMP2 and TRAPPC2) have a role in extracellular matrix (ECM) regulation, which is critical for processes required for successful pregnancy development (tissue remodelling, cell adhesion and migration). The allelic expression of TIMP2 gene, previously speculated to be imprinted in placenta, showed biased maternal expression in a subset of miscarriages and control elective terminations suggesting preferential maternal expression in early pregnancy. Finally, exome sequencing confirmed one autosomal recessive variant in an imprinted gene, RTL1. This gene is highly expressed in both the fetus and placenta and paternally imprinted.

Conclusions:
Our data showed that rare CNVs have a higher gene density than common CNVs and, as expected, could be more pathogenic. Additionally, we found that some rare CNV genes are involved in essential pathways during implantation and early embryonic development including ECM functioning and remodelling. Moreover, imprinted genes such as TIMP2 and RTL1 were affected by CNVs and sequence mutations, respectively, which could potentially affect pregnancy. In conclusion, new genomic tools, such as chromosome arrays and exome sequencing improve our understanding of the causes of pregnancy loss.

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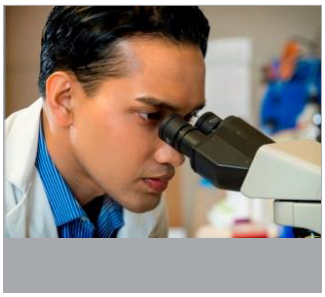

JAY GUNAWARDANA

Supervisor: Dr. Christian Steidl
Session: Clinical Sciences

"Frequent and somatic mutations of PTPN1 in primary mediastinal B cell lymphoma and Hodgkin lymphoma"

AUTHORS:
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Background/objectives:
Hodgkin Lymphoma (HL) accounts for 11% of all lymphomas and despite being one of the most curable lymphomas, 20% of HL patients still ultimately die of their disease. Similarly, a proportion of cases of primary mediastinal B cell lymphoma (PMBCL) frequently relapse early. Development of targeted therapeutic approaches is impeded by the lack of knowledge about the mutational landscape in the cancer genomes of these lymphomas. PTPN1 is a protein tyrosine phosphatase gene that encodes the protein, PTP1B. PTP1B dephosphorylates tyrosine residues on activated kinases to maintain cellular homeostasis. As overactive receptor kinases are critical oncogenic events in cancer, we hypothesized that constitutively active Janus kinase-Signal transducer and activation of transcription (JAK-STAT) observed in HL and PMBCL are in part due to a mutated PTPN1 gene with an impaired functional ability to dephosphorylate this constitutive signaling pathway.

Methods and Samples:
To investigate somatic PTPN1 mutations in HL and PMBCL, lymph node biopsies from 77 PMBCL and 30 HL patients were selected from tissue archives. DNA from PMBCL samples, microdissected Hodgkin Reed Sternberg (HRS) cells and 12 lymphoma-derived cell lines were extracted for PTPN1 exonic PCR amplification and Sanger sequencing. PTPN1 was silenced in a HL cell line (KMH2) by lentiviral transduction of a vector expressing shRNA. WT and mutant PTPN1 cDNA were cloned into a mammalian expression vector and expressed in HEK-293 cells. Protein expression of clinical samples, silenced and expressed cells were analyzed by immunohistochemistry and protein blotting.

Results:
After exclusion of reported SNPs and silent mutations, 19 PTPN1 coding sequence mutations were found in our PMBCL cohort, corresponding to 17 mutations (22%) in clinical samples and 2 in PMBCL-dervied cell lines. Twelve more mutations were discovered in our HL cohort, corresponding to 6 mutations (20%) in HRS cell samples and an additional 6 in HL-derived cell lines. In total, 18 (60%) missense, 4 (13.3%) frameshift, 3 (10%) single amino acid deletions, 4 (13.3%) nonsense mutations, and 1 (3.3%) promoter mutation were observed. Eight of these mutations were confirmed as somatic by sequencing of matched constitutional DNA. Silencing of PTPN1 resulted in hyperphosphorylation of JAK-STAT proteins and up-regulation of the oncogenes, MYC and BCL6. Expression of nonsense and missense PTPN1 mutants in HEK-293 cells led to sustained phosphorylation of STAT6 compared to empty vector (densitometric values Q9* 1.4, R156* 1.3, A69V 1.8, M74L 1.1, V184D 1.8 and M282L 1.1). No phosphatase activity was observed for the nonsense mutants and moderate phosphatase activity for the missense mutants using a tyrosine phosphatase-specific substrate (% activity compared to WT: Q9* 3.3%, R156* 3.7%, A69V 22.1%, M74L 79.7%, V184D 31.3% and M282L 79.6%). Immunohistochemical analysis showed PTPN1 mutations correspond to decreased protein expression in PMBCL (n=42; P=0.01) and in HL (n=30; P=0.04).

Conclusions:
PTPN1 is recurrently mutated in PMBCL and HL. These data suggest PTPN1 mutations as novel driver alterations in these lymphomas with implications for future treatment strategies.

CHARLES SOONG

Supervisor: Dr. Sam Aparicio
Session: Basic Sciences

"Development of a next-generation sequencing-based platform to study DNA double-strand break repair mechanisms"

AUTHORS:
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Background/objectives:
Cancers are caused by somatic mutations. While advances in next-generation sequencing technologies now allow us to possibly identify all the distinct mutational patterns, or signatures, within cancer genomes, very few of them have known underlying etiology, making it difficult to link these findings to practical treatment options. Defective DNA repair has previously been described in potentially generating some of the known or uncharacterized mutational signatures. Therefore, there is an interest in establishing a model system to study the DNA repair signatures to allow us to link the observed mutational signatures and the causal defects in cancer genomes. One type of DNA repair pathway is double-strand break (DSB) repair. Currently, two main mechanisms are known for DNA DSB repair: Non-homologous-end-joining (NHEJ) and homologous recombination (HR). The pDR-GFP (direct repeat - green fluorescent protein) construct is widely used as an image-based tool to study HR following DSB at a unique I-SceI restriction site (Pierce et al., 1999). Successful repair by HR results in a replacement of the I-SceI sequence with a BcgI sequence, yielding a correct GFP open reading frame and an observable GFP signal measurable by flow cytometry. We sought to integrate the pDR-GFP construct and establish a platform using NGS to directly analyze the repaired sites and measure the NHEJ and HR events.

Methods:
pDR-GFP expressing stable cell lines, DR-HCT116 (colorectal cancer) and DR-U2OS (osteosarcoma) were transfected with 20 nM siRNA. After 24 hr, DNA DSB was induced through I-SceI plasmid transfection, and genomic DNA was extracted 48 hours later. Repair site-specific 500bp amplicons were generated for each sample conditions. Barcoded NGS libraries were then generated and pooled for Illumina MiSeq sequencing. Obtained sequences were then aligned to the original pDRGFP sequence. Flow cytometry was also performed to compare the results with the NGS-based method.

Results:
Each potential outcomes of DNA repair resulted in a unique alignment signature. Uncut and NHEJ events aligned to the I-SceI region, with insertions and deletions in the case of NHEJ. HR events resulted in sequence reads aligned to a region containing a BcgI restriction site, corresponding to a sequence present at the wildtype open reading frame of GFP. %HR was quantified using the following formula: %HR = #reads aligned to BcgI / #reads aligned to BcgI + I-SceI. Overall, the %HR values obtained from NGS reads were higher, and showed more dynamic range than the %GFP values from flow cytometry. siRNA-mediated knockdown of RAD51 and BRCA2 - known genes in HR reproducibly led to a decrease in %HR from 75.4% in non-targeting control to 19.5% and 39.0%, respectively.

Conclusions:
Collectively, these data suggest that the NGS read alignment quantification-based approach provides a more sensitive method to reproducibly identify and quantify the DNA DSB pathways. Changes in the frequency of each DSB pathway can also be measured, at high confidence, following gene silencing of DNA repair genes.

CHRISTA KLEIN-BOSGOED

Supervisor: Dr. Dana V. Devine
Session: Basic Sciences

"Pathogen reduction technology impacts platelet mRNA: reduction in GPIIIa mRNA amount does not affect protein expression"

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Background/objectives:
The risk of bacterial contamination and the quality deterioration during storage reduces the shelf-life of platelet concentrates (PCs) to five days. Pathogen reduction technologies (PRTs) use UV irradiation with and without photosensitizers to inactivate pathogens in PCs by targeting DNA and RNA of viral or bacterial contaminants. Although platelets lack a nucleus and have no innate DNA transcription, they contain RNA and the ribosomal machinery to synthesize proteins. So far, however, little is known about the role of protein synthesis in platelets. PRTs are known to accelerate platelet storage lesion, but it is unknown whether this affects platelet mRNA and subsequent translation.

Methods:
Apheresis PCs were collected from volunteer donors according to Canadian Blood Services standard procedures. In a pool-and-split design, units were treated with Mirasol® PRT system (TerumoBCT) that uses riboflavin and UV light or were left untreated. Glycoprotein (GP)IIIa, a subunit of the platelet fibrinogen receptor, was used as a model protein as it is known to be translated during storage. Samples were drawn during storage and RNA was extracted using TRIzol® (Invitrogen). Quantitative Polymerase Chain Reaction (qPCR) was performed using primers specific to glycoprotein GPIIIa to evaluate mRNA expression. Receptor expression was monitored by flow cytometry and lysates were analyzed by western blot.

Results:
Reduction of the relative amount of GPIIIa transcripts was observed immediately after treatment. Interestingly, the degradation rate of the mRNA remaining after PRT treatment is lower, resulting in a longer half-life for GPIIIa mRNA compared to untreated control platelets. However, no significant difference was observed in GPIIbIIIa protein expression during storage for PRT treated and control platelets. The expression of the GPIIIa subunit, measured by flow cytometry was increased in PRT treated platelets and decreased during storage for both PRT treated and control platelets. Fibrinogen bound to receptor complexes on the surface increased during storage in PRT treated platelets, while this level remained consistent for the control platelets. The response to ADP, measured by affinity for PAC-1 antibody which specifically recognized active fibrinogen receptor, decreased during storage and was similar in both PRT treated and control platelets.

Conclusions:
Although the mRNA level is decreased upon PRT treatment, the half-life of the mRNA transcript of GPIIIa is longer than for the untreated platelets. This observation might be explained by a mechanism protecting mRNA from degradation. Even though mRNA transcript amount was reduced, no change in protein expression was observed after PRT treatment. The membrane receptor expression was increased in PRT treated platelets, suggesting increased levels of activation. Protein levels do not necessarily correlate with mRNA levels, suggesting increased translational activity of specific proteins. Although the significance of platelet mRNA is unknown, it is another aspect of platelet physiology that is affected by exposure to PRT.

EMMA CONWAY

Supervisor: Dr. Wan L. Lam
Session: Basic Sciences

"Characterization of macrophage populations in the lungs of healthy individuals and cancer patients "

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Lung cancer is the leading cause of cancer mortality, accounting for an estimated 20,100 Canadian deaths in 2012, or 27.3% and 26.0% of cancer deaths in men and women, respectively. At only 18%, lung cancer has one of the lowest 5-year survival rates of all malignancies. This is largely attributed to late stage diagnosis and ineffective treatment strategies, highlighting the need for novel, more effective treatments. Within the past decade, the role of the immune system in tumorigenesis has become increasingly appreciated, such that immune evasion is now considered one of the hallmarks of cancer. Macrophages are the predominant immune cell type in the lung and lung tumors, and their presence in tumors – tumor associated macrophages (TAMs) – has been associated with progression and aggressiveness. It is widely accepted that a spectrum of macrophage activation states exists; at opposite ends of this spectrum there exist M1 macrophages which are pro-inflammatory and have anti-tumor functions, and M2 macrophages which are anti-inflammatory and promote tumorigenesis. An understanding of the baseline immune cell composition and phenotypes of macrophages in healthy and diseased lungs is necessary for further investigation into the roles these cells play in tumor development, progression and response to treatment.

Methods:
Bronchoalveolar lavage (BAL) washes were collected from the lungs of patients with or without lung cancer. Washes were obtained from the lobe containing the tumor as well as from a healthy lobe. Detailed clinical information and CT images are available for all patients. Macrophages were quantified and sorted by multi-colour flow cytometry using a panel of cell surface protein markers, developed specifically for the analysis of human samples, and the BD FACSAria III Cell Sorter.

Results:
Comparison of patient samples and in vitro skewed macrophage controls revealed that macrophages from diseased lobes are skewed towards an M2-like phenotype, while macrophages from the lobes of healthy former smokers are largely unskewed, with low expression of M1 and M2 markers on the majority of macrophages.

Conclusions:
Our findings suggest that differences in macrophage polarity exist between healthy and diseased lobes; however, whether these differences precede tumor development remains to be discerned. In vitro functional assays of sorted macrophages and analysis of additional samples, especially precursor lesions such as dysplasias, will be instrumental in determining how macrophage polarity influences tumorigenesis.

ALISTAIR CHENERY

Supervisor: Dr. Colby Zaph
Session: Basic Sciences

"Overlapping mucosal immunity: regulation of allergic lung inflammation by intestinal whipworms"

AUTHORS:
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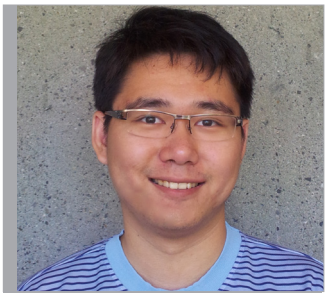
Background/objectives:
The prevalence of allergic disease is incredibly striking in the Western world. Asthma in particular is major health concern for Canada with over 3 million people diagnosed with the disease. Environmental factors are known to play a strong role in determining the risk for asthma. For instance, the global incidence of parasitic worm infections tends to be inversely associated with the occurrence of asthma. Indeed, parasitic worms have a powerful ability to manipulate the host immune system and there is evidence that they can directly protect against asthma. There is also an emerging concept of mucosal immune crosstalk between the intestine and the lungs in which intestinal worms may promote protection from allergic lung disease. The objective of this study is to examine the gut-lung cellular and molecular crosstalk of the host during intestinal worm infections in the context of lung disease. Our hypothesis is that chronic infections with intestinal whipworms protect against asthma through this gut-lung immunological axis.

Methods:
C57BL/6 mice were chronically infected with the intestinal whipworm Trichuris muris by oral gavage of infective eggs. Following infection, mice were sensitized and challenged intranasally with protease allergens to induce allergic lung inflammation. Lung disease was assessed by bronchoalveolar lavage fluid analysis by flow cytometry, QRT-PCR of lung tissue, and lung histopathology. ELISA was performed to measure ex vivo cytokine secretion. Experiments were also performed using mice depleted of CD4+ T cells and immunodeficient Rag1-/- mice that lack an adaptive immune system.

Results:
Mice chronically infected with T. *muris* were protected from allergic lung inflammation. Infection significantly blocked airway cellular infiltration, eosinophilia, and mucus hyperproduction after experimental induction of asthma. Infection also caused a mixed immune response in the lung characterized by increased interferon-gamma and interleukin-10 that are known to be protective against allergic lung inflammation. Interestingly, T. *muris* exerted effects on both innate cell and adaptive CD4+ T cell populations indicating that there are multiple immune pathways in which T. *muris* can protect against allergic lung disease.

Conclusions:
Our results show that an intestinally-restricted helminth can exert protective immune effects that can suppress the development of allergic lung inflammation. This confirms that there is an immunological link between two anatomically distinct mucosal sites. Future studies aim to investigate cellular trafficking between the gut and the lung that may be mediate protection from asthma during intestinal worm infection. These results are relevant for both studying host-parasite relationships as well as for defining novel immune pathways that may have therapeutic potential for the treatment of asthma.



ANTHONY HSIEH

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Measuring the effects of chronic HIV infection and other clinical modulators on cellular aging in senescent cytotoxic T cells"

AUTHORS:
Anthony Hsieh[1], Hélène Côté[1] for the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

AFFILIATIONS:
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Background/objectives:
Combination antiretroviral therapy (cART) has proven an effective measure for increasing life expectancy and preventing the onset of AIDS in people living with HIV. However, even cART-treated people are still at higher risk for age-related comorbidities earlier in life including cardiovascular disease, non-AIDS-related cancers, and liver disease compared to HIV-uninfected people. Small studies have shown that persistent age-related immunologic abnormalities such as an expanded senescent cytotoxic T lymphocyte compartment (CD8+CD28- CTLs) and a decreased CD4:CD8 ratio exist in cART-treated people living with HIV. It has not been established that these age-related abnormalities predict comorbidities related to HIV. It is also unclear whether an association between chronic HIV infection and skewed cellular aging markers such as telomere length and mitochondrial damage in the specifically expanded senescent CTL compartment exists. Furthermore, it has yet to be determined how HIV clinical history is related with these aging markers in the immune compartment. This pilot study seeks to better characterize cellular aging in the senescent CTL compartment in people living with HIV to gain insight on the mechanism behind early onset comorbidities. We hypothesize that HIV clinical parameters such as viral load and time since infection are associated with cellular aging markers in the immune system.

Methods:
For this pilot study, whole blood was collected from 43 individuals from ages 6 to 70 enrolled in the **C**hildren and Women: **A**nti**R**etrovirals and **M**arkers of **A**ging (CARMA) cohort study, including 10 HIV- controls. Flow cytometry and cell sorting was used to separate senescent CTLs from mononuclear cells isolated from peripheral blood. A qPCR-based assay previously used to measure leukocyte telomere length was used to calculate telomere length in the senescent CTL subset. A separate qPCR-based assay was employed to measure apparent oxidative damage in mitochondrial DNA. Statistical analyses were performed using Spearman and Mann-Whitney U tests in the IBM SPSS and XLSTAT software. For some experimental and clinical parameters, data was not yet collected in all participants.

Results:
This study suggested significant correlations between several measured aging markers and clinical variables. As expected, HIV infection status was associated with a lower ratio of CD4+:CD8+ T cells ($p=0.001$) as well as lower CD4+ and higher CD8+ T cell counts individually ($p=0.001$ and $p=0.004$), while HIV viral load was correlated with CD8+ T cell count ($p=0.022$, $R2=0.398$, $n=33$). However, HIV viral load was negatively correlated with B cell count ($p=0.049$, $R2=-0.346$, $n=33$). Mitochondrial oxidative damage in naïve CD8+ T cells was correlated with time since HIV infection ($p=0.015$, $R2=0.81$, $n=8$). A correlation was also seen between mitochondrial oxidative damage and telomere length in senescent CD8+ T cells ($p=0.009$, $R2=0.77$, $n=10$) highlighting a link between the two cellular aging markers.

Conclusions:
CART does not completely restore health in people living with HIV. This study exposes several clinical factors such as duration of HIV infection and viral load as potential predictors of immune cell aging. This pilot will lead to a larger project involving a larger sample size and multivariate analyses. Better understanding the interaction between HIV, cART, and new predictors for age-related comorbidities is the crucial next phase in advancing treatment for people living with HIV.

KYLE BURROWS

Supervisor: Dr. Colby Zaph
Session: Basic Sciences

"The transcriptional repressor Hic1 modulates T cell fate and function"

AUTHORS:
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Background/objectives:
Naïve CD4+ T helper (T_H) cells can differentiate into several distinct subsets that have specific functions in vivo. T_H cell lineage choice is a complex process that requires a balance between transcriptional activation of lineage specific cytokines, transcription factors and the silencing of lineage-promiscuous genes. The BTB/POZ family of transcriptional repressors includes Bcl6 and Thpok, which play critical roles in TH cell differentiation and function. Hypermethylated in cancer 1 (Hic1) is a member of this family and can be up regulated in TH cells upon stimulation with retinoic acid (RA), a vitamin A metabolite. RA has also been shown to promote the acquisition of gut-specific lymphocyte adhesion/trafficking molecules. However, no role for Hic1 in T_H cells has been examined. Our hypothesis is that Hic1 is critically required for the differentiation and function of T_H cells.

Methods:
Naïve CD4+ T cells were isolated from spleens and lymph nodes of C57Bl/6 mice and mice with a specific deletion of Hic1 in T cells (Hic1ΔT mice) and polarized into different T_H cell subsets. Using a retroviral vector containing the HIC1 gene, an IRES and GFP, we retrovirally over expressed Hic1 in T_H cells. To monitor Hic1 expression *in vivo*, we utilized a novel reporter mouse (Hic1-CreERT2/tdTomato) where the fluorescent protein Tomato would only be expressed upon both tomoxifen treatment and expression of Hic1. Flow cytometry and qRTPCR were used to assay expression of T_H effector cytokines and transcription factors.

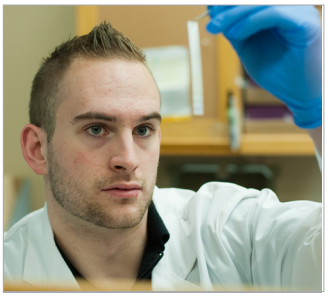
Results:
In vitro, Hic1 expression was low in naïve T_H cells, was specifically induced in T_H17 and regulatory T (Treg) cells and Hic1-deficient T_H cells displayed dysregulated T_H17 and Treg cell differentiation. Under T_H17 conditions, Hic1-deficient T_H cells exhibited heightened T_H17 differentiation, with increased expression and production of IL-17, despite normal levels of key T_H17 cell-promoting genes including RORγt, Batf and Stat3. In contrast, Hic1-deficient T_H cells failed to efficiently differentiate into Foxp3+ Treg cells. Thus, Hic1 regulates the balance between TH17 and Treg cell differentiation. Hic1 directly interacted with phosphorylated Stat3 in T_H17 cells, providing a potential mechanism for transcriptional repression of Stat3-dependent gene expression. In addition, retroviral overexpression of Hic1 in T_H17 cells inhibited IL-17 production. *In vivo*, Hic1 was expressed specifically in CD4+ T cells in the intestine but not in peripheral lymphoid tissues such as the spleen. Furthermore, CD4+ T cell-intrinsic Hic1 deficient mice lack CD4+ T cells in the gut in the steady state, demonstrating a crucial role for Hic1 in the homeostasis of intestinal T cells.

Conclusions:
Taken together, these results establish a T cell-intrinsic role for Hic1 in T_H17 and Treg lineage differentiation and identify a critical function for Hic1 in regulating T cell activity in the intestine.

JESSE OLSON

Supervisor: Dr. Angela Devlin
Session: Basic Sciences

"Developmental exposure to maternal folic acid and vitamin B12 imbalance programs pancreatic islet mafa expression and glucose tolerance in female offspring mice"



AUTHORS:
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Background/objectives:
Risk for cardiometabolic disease may be programmed or accelerated by prenatal or early postnatal nutritional imbalances. For example, population studies have reported greater adiposity and insulin resistance in children from mothers with high folic acid (FA) intakes and low vitamin B12 (B12) status during pregnancy. In Canada its been reported that women of child-bearing age have adequate folate status but 5% may be vitamin B12 deficient during the early stages of pregnancy. Currently, little is known about the effect of maternal high FA intakes on a background of low B12 status on the offspring and presentation of cardiometabolic disease in adulthood.
Objective: To investigate the molecular mechanisms underlying the programming of adiposity and glucose metabolism by developmental exposure to maternal FA and B12 imbalance.

Methods:
Female C57BL/6J mice were fed one of the following diets during pregnancy and lactation: high folic acid with adequate B12 (HFA+B12), high folic acid deficient in B12 (HFA-B12), or a control diet. At weaning, female offspring mice were fed either a control diet or Western diet for 30 weeks. Body composition was assessed by EchoMRI and surgical dissection of adipose tissue depots. Glucose tolerance tests were conducted at 20 and 30 weeks post weaning. Beta cell mass was quantified by immunofluorescence. Expression of Pdx1, Mafa, Ins1, and Ins2 mRNA was quantified by real-time PCR in pancreatic islets.

Results:
At 20 wks post weaning, offspring mice fed the control diet that were from dams fed the HFA-B12 diet had greater glucose intolerance ($P<0.05$) than offspring from dams fed the control or HFA+B12 diets. However this was not observed at 30 wks post weaning despite lower ($P<0.05$) beta cell volume in offspring from dams fed the HFA-B12 diet. Interestingly, offspring mice from HFA-B12 diet fed dams had greater ($P<0.05$) islet Mafa mRNA expression than offspring from control or HFA+B12 diet fed dams.

Offspring fed the post weaning western diet from females fed the HFA-B12 diet had lower ($P<0.05$) fasting blood glucose concentrations than offspring from control diet fed dams and lower ($P<0.05$) fasting insulin concentrations than offspring from control and HFA+B12 diet fed dams. This was accompanied by greater ($P<0.05$) beta cell mass in offspring from HFA-B12 diet fed dams compared to offspring from dams fed the HFA+B12 diet.

Conclusions:
These findings provide evidence that programming of glucose intolerance in adult females by maternal FA/B12 imbalance may involve direct effects on pancreatic beta cells and is influenced by diet during adulthood.

MELISSA GLIER

Supervisor: Dr. Angela Devlin
Session: Basic Sciences

"The role of cystathionine-beta-synthase in obesity-related cardiac lipotoxicity"

AUTHORS:
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AFFILIATIONS:
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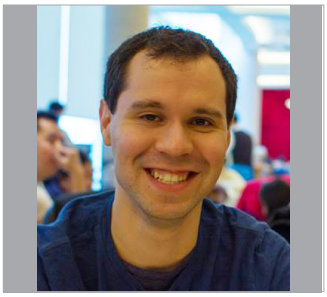
Background/objectives:
Obesity-related cardiac lipid accumulation is associated with altered cardiac energy metabolism, lipotoxicity and dysfunction. The molecular mechanisms underlying obesity-related cardiac lipid accumulation and how this causes cardiac dysfunction is not well understood. Cysteine is a sulfur-containing amino acid required for the synthesis of the antioxidant glutathione. Cysteine can be synthesized from the transsulfuration of homocysteine by cystathionine-beta-synthase (CBS). We previously reported mice heterozygous for targeted disruption of Cbs (+/-) with diet-induced obesity have enhanced cardiac lipotoxicity and greater glucose intolerance. The goal of this study is to determine whether Cbs+/- mice with diet-induced obesity have impaired cardiac function and to explore mechanisms underlying the augmented lipotoxicity in this model.

Methods and Results:
Cbs+/+ and Cbs+/- mice were fed a high-fat diet (HFD) from weaning for 20 weeks to induce obesity and glucose intolerance. As expected, Cbs+/+ and Cbs+/- mice fed the HFD had greater ($P<0.001$) final body weights, visceral (retroperitoneal and epididymal) and subcutaneous (inguinal) fat pad weights compared to mice fed chow diet. Interestingly, Cbs+/- mice had smaller ($P<0.05$) visceral fat pads compared to Cbs+/+ mice, but only in those fed the chow diet. Mice fed the HFD had greater ($P<0.01$) contractile function measured by ejection fraction and fractional shortening, as well as greater ($P<0.01$) interventricular septum thickness compared to chow fed mice. Interestingly, Cbs+/- mice independent of diet, had larger ($P<0.01$) hearts compared to Cbs+/+ mice. This was accompanied by higher ($P<0.01$) triglyceride, arachidonic acid (AA) and docosahexaenoic acid (DHA) concentrations in hearts from Cbs +/- mice compared to Cbs+/+ mice. Mice fed the HFD had higher ($P<0.01$) AA but lower ($P<0.001$) DHA concentrations in heart compared to mice fed chow diet. Cbs+/- mice had higher mRNA expression of genes involved in fatty acid uptake (CD36, $P<0.01$), oxidation (carnitine palmitoyltransferase 1, $P<0.01$), and triglyceride synthesis (diacylglycerol acyltransferase 1, $P<0.01$) in the heart compared to Cbs+/+ mice. We further quantified AMP activated protein kinase alpha (AMPKa) expression, a regulator of cellular energy homeostasis which has also been implicated in the development of cardiac hypertrophy. Cbs+/- mice had a lower ($P<0.01$) ratio of phosphorylated-AMPKa/AMPKa expression in the heart than Cbs+/+ mice. Yet, mice fed the HFD had a lower ($P<0.001$) ratio of phosphorylated-AMPKa/AMPKa expression in heart compared to mice fed chow diet, and this occurred to a greater extent in Cbs+/- mice.

Conclusions:
Collectively, these findings suggest that the cardiac lipotoxicity in Cbs+/- mice with diet-induced obesity is associated with disturbances in cardiac fatty acid metabolism, cardiac hypertophy and cardiac dysfunction. These findings are the first to demonstrate a novel role for CBS in the pathology of cardiac lipotoxicity.

ABSTRACT # 27



JAQUES COURTADE

Supervisor: Dr. Bruce Verchere
Session: Basic Sciences

"Impaired processing of human pro-islet amyloid polypeptide (proIAPP) promotes early islet graft failure"

AUTHORS:
Jaques A. Courtade[1], Paul C. Orban[1], C. Bruce Verchere[1]

AFFILIATIONS:
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Background/objectives:
Islet transplantation is a promising therapy for type 1 diabetes that involves the transplantation of healthy insulin-producing beta cells to achieve normoglycemia and insulin independence. Long-term survival of islet grafts may be compromised not only by immune rejection but also other factors including the formation of islet amyloid from islet amyloid polypeptide (IAPP). IAPP aggregation has been linked to beta-cell dysfunction, apoptosis, and islet inflammation but the underlying mechanism of islet amyloid formation remains elusive. Elevated circulating proinsulin:insulin ratios have been detected in type 2 diabetes and in type 1 diabetic recipients of islet transplants. Processing of the IAPP precursor, proIAPP, may be similarly compromised in islet transplants and type 2 diabetes, since like proinsulin, proIAPP is processed by the beta cell prohormone convertases PC1/3 and PC2. We hypothesize that accumulation of proIAPP intermediates leads to amyloid formation and beta-cell dysfunction and assessed the impact of impaired proIAPP processing in transplanted islets.

Methods:
NOD.SCID mice (age 8-10 weeks) were made diabetic with streptozotocin and transplanted with islets from mice with beta-cell expression of human proIAPP and lacking PC2 (hIAPPTg/0; PC2-/-) or controls (hIAPPTg/0;PC2+/-, hIAPP0/0;PC2-/-, hIAPP0/0;PC2+/-). Blood glucose was monitored weekly in recipients for 16 weeks or until graft failure (return of hyperglycemia). Islet graft sections were stained for insulin, amyloid deposition (thioflavin S) and TUNEL positivity.

Results:
Diabetes (blood glucose >16.9 mM) returned in almost all (90%) of recipients of hIAPPTg/0;PC2-/- islets within 16 weeks post-transplant, whereas recipients with normal human proIAPP processing (hIAPPTg/0;PC2+/-) remained normoglycemic for the entire 16 weeks. Recipients of grafts expressing native rodent IAPP, with or without PC2, similarly maintained normoglycemia. Despite early graft failure in recipients of islets with impaired human proIAPP processing, beta-cell mass remained unchanged and amyloid deposition was lower compared to human proIAPP-expressing mice with normal processing. Islet grafts had few TUNEL-positive beta cells and no difference in the number of TUNEL-positive cells among groups, suggesting that impaired human proIAPP processing compromises beta-cell function rather than promoting beta-cell apoptosis.

Conclusions:
Impaired human proIAPP processing in islet transplants induces early graft failure without affecting beta-cell mass or increasing amyloid formation. These data suggest that impaired proIAPP processing promotes dysglycemia, possibly via the formation of early (pro)IAPP aggregates that induce beta-cell dysfunction. Our work implicates IAPP precursors as potential targets to prolong islet graft survival.

YUDA SHIH

Supervisor: Dr. Catherine Pallen
Session: Basic Sciences

"Oligodendroglial protein tyrosine phosphatase alpha signaling is altered by Wnt and promotes myelination in glial-neuron co-cultures"

AUTHORS:
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Background/objectives:
Myelin is a fatty material that ensheathes and insulates nerve fibers to promote efficient conduction of nerve signals and protect nerves from damage. Specialized glial cells called oligodendrocytes (OLs) carry out myelination in the central nervous system (CNS). OLs differentiate from precursor cells (OPCs) into their mature myelinating form. Multiple sclerosis (MS) is a demyelinating disease affecting the CNS that causes neurological deficits resulting from myelin destruction. Remediating acquired myelin defects in MS through myelin repair is a key therapeutic goal in treating this debilitating disease; however, the molecular mechanisms regulating myelination remain poorly understood. We have previously shown that the transmembrane-like receptor protein tyrosine phosphatase alpha (PTPα) is an important regulator of OPC differentiation. However, the role of PTPα in myelination remains unclear. The canonical Wnt signaling pathway has been shown to be involved in OPC differentiation and myelination. Loss of PTPα and stimulation of canonical Wnt signalling share similarities, with both maintaining OPCs in a proliferative immature stage. Here, we established a neuron-glia co-culture model system to investigate the role of PTPα to promote not only differentiation but also myelination. Furthermore, we investigated whether Wnt signalling can alter PTPα signalling during OPC differentiation and myelination.

Methods:
Primary murine neural stem cells were isolated from cerebral cortices of E14-17.5 mice and cultured in the presence of growth factors to generate OPCs. Oligodendrocyte-neuron co-cultures were established using dorsal root ganglion neurons (DRGNs) isolated from spinal cords of postnatal day 5-7 mice and grown for 14 days on laminin 2/4-coated chamber slides. Wild-type and PTPα KO OPCs were then introduced to the DRGNs and co-cultures were maintained for another 14 days during which co-cultures were either 1) untreated, 2) treated with 100ng/mL Wnt3a to stimulate the Wnt signaling pathway and 3) treated with varying concentrations of XAV939, a Wnt signaling pathway inhibitor. The co-cultures were immunostained for neurofilament (NFH) to label the neurite bed and for myelin basic protein (MBP), a mature OL marker.

Results:
Imaging and quantitative analysis using ImageJ allowed the determination of neurite bed density (NFH signal), MBP expression, and MBP/NFH co-localization as a indicator of potential myelination. We observe that MBP/NFH co-localization is significantly enhanced by ~ 2-fold with WT OPCs compared to KO OPCs (% MBP/NFH co-localization on laminin 2/4: WT OPC 16.8 ±3.5% vs. KO OPC 8.4 ±1.6%, p=0.009, n=3). We also find PTPα-dependent effects on MBP/NFH co-localization can be altered by promoting or inhibiting Wnt signaling. The defective co-localization with PTPαKO OPCs can be restored to levels equivalent to that observed with WT OPCs by treating the co-cultures with XAV939. Conversely, stimulating Wnt signaling by treating co-cultures of WT OPCs with Wnt3a inhibits MBP/NFH co-localization by about 50% to levels equivalent to that observed with PTPα KO OPCs.

Conclusions:
Our results indicate that PTPα is required for OPC differentiation and possibly myelination in co-cultures. Furthermore, these findings suggest that signaling actions of PTPα in OPC differentiation can be regulated by, and may crosstalk with, Wnt signaling. Collectively, delineating the precise mechanisms by which Wnt signaling and PTPα signaling regulate OPC differentiation and myelination may provide insights to develop therapeutic strategies for treating demyelinating disorders.

SOPHIE STUKAS

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Intravenously injected human apolipoprotein A-I rapidly enters the central nervous system via the choroid plexus in mice"

AUTHORS:
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AFFILIATIONS:
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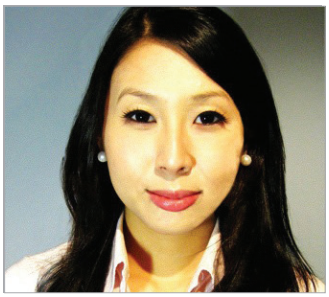
Background/objectives:
Cerebrovascular dysfunction contributes significantly to the pathoetiology of Alzheimer's disease (AD). Midlife vascular risk factors, such as hypertension, cardiovascular disease, diabetes, and dyslipidemia, increase the relative risk for AD. These comorbidities are all characterized by low and/or dysfunctional high-density lipoproteins (HDL), which itself is a suggested risk factor for AD. In addition to lipid transport, HDL enhances vasorelaxation, reduces inflammation and oxidative stress, and promotes endothelial cell survival and integrity. In mouse models of AD, apolipoprotein (apo) A-I, the primary protein component of circulating HDL, reduces neuroinflammation, suppresses cerebrovascular amyloid deposition, and protects cognitive function, making it an intriguing therapeutic target. However, how apoA-I, which is made only in the liver and intestine yet is present in cerebrospinal fluid (CSF) and brain tissue lysates, enters the central nervous system (CNS) is unknown.

Methods:
Steady state levels of murine apoA-I in CSF and brain tissue were determined by denaturing immunoblot and expressed relative to plasma and liver, respectively. To determine the relative CNS biodistribution and pharmacokinetics of apoA-I, we performed intravenous injections of recombinant, fluorescently-tagged lipid poor human (h) apoA-I in mice. We performed two main studies: a dose response of 7.5 - 120 mg/kg at 2h, and a timecourse, where mice were injected with 60 mg/kg and analyzed 0.5 - 24h post injection. HapoA-I levels in plasma, liver, kidney, brain and CSF were determined by ELISA. CNS distribution was evaulated using immunohistochemistry and confocal microscopy. We performed further studies using primary human epithelial cells derived from the choroid plexus to exaime hapoA-I binding and transport.

Results:
Steady state levels of murine apoA-I in CSF and brain are approximately 0.01% and 10-15% of its levels in plasma and liver, respectively. HapoA-I injected intravenously into mice localizes to the choroid plexus within 0.5h and accumulates in a saturable, dose-dependent manner in the brain. HapoA-I accumulates in the brain for 2h, after which it is eliminated with a half life of 10.3h such that 10% of maximal observed levels are remaining 24h after injection. *In vitro*, hapoA-I is specifically bound, internalized and transported across confluent monolayers of primary human choroid epithelial cells.

Conclusions:
Following intravenous injection, hapoA-I rapidly localizes predominantly to the choroid plexus. As apoA-I mRNA is undetectable in murine brain, our results suggest that apoA-I is expressed in the liver and intestine, secreted into plasma, and then gains access to the CNS primarily by crossing the blood-CSF barrier (BCSFB) via a specific receptor mediated mechanism.



SOPHIA WONG

Supervisor: Dr. Andre Mattman
Session: Clinical Sciences

"Total IgG method insensitivity to the presence of IgG4 immunoglobulins"

AUTHORS:
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Background/objectives:
Immunoglobulin G4-related disease (IgG4-RD) is detected in part by measurement of serum IgG4 levels in patients suspected to have the condition. The immunonephelometric methods used to measure serum IgG4 are susceptible to hook effect errors. The quality control parameter, IgGRDiff = 100 * [IgG Sum(IgG1, IgG2, IgG3, IgG4) - Total IgG] / Average [IgG Sum(IgG1, IgG2, IgG3, IgG4), Total IgG], is theoretically of use in detecting a large IgG4 hook effect. We evaluated the IgGRDiff parameter in this context.

Methods:
Retrospective review of the IgGRDiff parameter was conducted in a cohort of patient samples (n=163). All specimens were tested with the Siemens BN II analyzer utilizing Siemens (total IgG measurement) and Binding Site (IgG subclasses measurement) reagents. The 95th percentile for the absolute value of IgGRDiff was determined and compared to the IgGRDiff values in samples with known hook effect (n=6). IgGRSum was calculated for each IgG subclass [for example, IgG4RSum = 100 * IgG4 / IgG Sum(IgG1, IgG2, IgG3, IgG4)], and IgGRDiff was regressed against IgGRSum for each subclass.

Results:
The 95th percentile of the absolute IgGRDiff parameter was 22.8% in our study sample set. In 4/6 cases with known hook effect, the absolute value of the IgGRDiff was <22%, indicating low sensitivity of IgGRDiff in identifying IgG4 hook effect. Further, IgG4RSum was directly correlated with IgGRDiff (IgGRDiff = 1.21 * IgG4RSum - 7.9; R^2=0.62, $p<0.001$), suggesting that the total IgG method has limited reactivity with IgG4 immunoglobulins.

Conclusions:
Siemens' Total IgG method underestimates the amount of immunoglobulins present when IgG4 levels are raised. This negative bias, in turn, affects the calculation of the IgGRDiff quality control parameter, and limits its ability to detect IgG4 hook effect errors.

ABHINAV AJAY KUMAR

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Investigation of infant leukocyte telomere length following exposure to maternal HIV plasma viral load and antiretroviral drugs during pregnancy"

AUTHORS:
Abhinav Ajay Kumar[1], Hélène FC Côté[1], 2 for the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

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Background/objectives:
More than 30 million people live with HIV globally, of which 48% are women, and almost 10% are children. Current antiretroviral therapies (ART) have been highly successful in preventing mother-to-child-transmission in the 1.5 million children born to HIV+ mothers each year but there are concerns that exposure to ART in utero could have long-term effects on these HIV-exposed uninfected (HEU) children. Many ART drugs can cross the placenta and could affect the developing fetus, but so could exposure to maternal HIV "milieu". In addition, HEU infants receive ART for the first 6 weeks of life. Leukocyte telomere length (LTL) reflects cellular replication history and is a marker of cellular aging, with short LTL being linked to cardiovascular disease and immunosenescence. HIV proteins and certain ART drugs inhibit telomerase, the enzyme that elongates telomeres. A previous study in our lab showed that HIV+ children (0-19y) not on combination ART (cART) had shorter LTL compared to those on cART, suggesting that uncontrolled HIV viremia rather than cART exposure may affect LTL. In addition, ART-exposed HEU infants (0-3d) did not have shorter LTL than control infants born to HIV- mothers, although a trend towards shorter cord blood LTL in HEU infants was observed. The timing of maternal cART in pregnancy, and therefore duration of exposure to HIV products and/or drugs could influence infant LTL.
Hypothesis: Infants born to HIV+ mothers who started cART late in pregnancy (2nd and 3rd trimesters), will have shorter LTL compared to infants with less HIV milieu exposure such as infants born to HIV+ mothers who conceived already on cART or started it during the 1st trimester, or control infants born to uninfected mothers.

Methods:
Study subjects included HEU infants (0-9 months) enrolled in the CARMA cohort study (n=221); many (n=110) with ≥2 samples collected during the first year of life, and unexposed controls (0-9m, n=152) with single samples. LTL was measured on whole blood DNA via monochrome multiplex qPCR. The T/S ratio (ratio of the Telomere (T) gene to Single copy nuclear (S) gene), a measure of relative LTL, was compared between HEU and controls.

Results:
Experimental data was collected for 85/221 HEU (with a total of 167 distinct blood samples) and 82 controls. Although analyses are not completed, no obvious differences in relative LTL were detected between groups at birth (0-10d). Similarly, there does not appear to be major longitudinal differences in LTL attrition rate over either the first 6 weeks or 9 months of life.

Conclusions:
The fact that no differences were seen is reassuring for the health of the infants. Future work will involve analyzing the remaining samples and extending the age range to 2 years to get a better determination of the attrition rates. The number of HEU infants born with extensive cART exposure is rising rapidly worldwide, and little is known on the effect(s) of such exposure. Our research will shed light on LTL dynamics in this context.

AHMAD ARBAEEN

Supervisor: Dr. Dana V. Devine
Session: Clinical Sciences

"The efficiency of Thromboelastography to discriminate good vs poor quality buffy coat platelet concentrates"

AUTHORS:
Ahmad F Arbaeen, Elena Levin, Katherine Serrano, and Dana V. Devine

AFFILIATIONS:
[1]Canadian Blood Services; [2]Center of Blood research, UBC. 3-Umm Al-Qura University in Saudi Arabia

Background/objectives:
Blood platelet transfusion is crucial to maintain hemostasis and compensate for blood loss in trauma patients. Although 4.2 million platelet transfusions (prophylactic or therapeutic) occur annually in North America as part of cancer therapy and treatment of bleeding complications, approximately 30% of these transfusions are failing to meet the desired clinical outcome as measured by Corrected Count Increment (CCI) 24 hours following transfusion. The failure to meet this desired clinical outcome has been primarily attributed to ineffective platelet concentrates (PCs) in addition to other factors. PCs are transfused without pre-transfusion characterization, and the outcome in the patient is the only indicator of platelet effectiveness. Thus there is a strong need for assays that can be applied to PCs to test the efficiency of clot formation and platelet activation in a manner that models these processes in the bloodstream prior to transfusion. We are specifically interested in determining whether the technology of thromboelastography (TEG), used in operating rooms to assess the hemostatic state of whole blood for patients susceptible to bleeding, can be applied to the quality assessment of platelet concentrate (PC). We hypothesize that application of TEG to evaluate the procoagulant activity of stored platelets can predict platelet quality and ultimately transfusion outcome. This study aims to distinguish between hyper, moderate, and non-responsive PCs and to prevent excessive bleeding by transfusing an efficient PC.

Methods:
After validating the TEG-PC assays, five buffy coat PCs were used to evaluate TEG responses in stored PCs. They were sampled on days 1, 5, and 10 and were reconstituted with fresh frozen plasma (FFP) to different platelet concentrations (400, 300, 200, 100, 40, and 25 x 109 cells/L). The maximum amplitude (MA) of clot strength was determined, which represents platelet activity and fibrinogen interaction. Cytochalasin D (CD), an inhibitor of actin polymerization, was used to determine the background MA: the clot strength of fibrinogen when blocking platelet activity. For instance, MA-platelet contribution= MA (PC) - MA (CD treaded PC). Purposely generated poor quality PCs were prepared and sampled on storage days 2, 5, and 8 and analyzed by TEG to generate a base line response for the "bad" platelets. In addition, other in vitro quality parameters, such as, pH, CD62P expression, and annexin A5-binding were measure as well.

Results:
In TEG, CD treated PC were able to give reproducible results of only fibrinogen contribution to MA, 30.4 mm, when platelet counts are between 100-400 x 109/L. The delta of MA showed a good contribution of platelets on TEG. TEG analysis showed no significant change in MA of fibrin formation during storage up to day 10 when platelet concentrations are 100 x 109 cells/L or higher. However, other in vitro quality parameters showed an elevation of the procoagulant phosphatidylserine level (determined by the annexin A5 binding) on the cell surface, as well as an increase in the P-selectin level (CD62P expression) during storage time. On day 8 of storage, the purposely generated poor qualities PC, showed a decrease in MA which reflects a significant decrease in the platelet quality.

Conclusions:
TEG-PC assay was evaluated to be suitable to confirm the in vitro functional quality of stored buffy coat PCs but needs further validation to make it sensitive to donor-to-donor variation. Correlation of the TEG-PC assay with in vivo clinical outcome should be also assessed preferably in hematological patients under PC prophylactic transfusion.

MOMIR BOSILJCIC

Supervisor: Dr. Kevin Bennewith
Session: Basic Sciences

"Myeloid-derived suppressor cell accumulation in secondary target organs promotes a higher metastatic potential in breast cancer "

AUTHORS:
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AFFILIATIONS:
[1]Integrative Oncology Department, BC Cancer Agency; [2]Department of Pathology and Laboratory Medicine, UBC; [3]Interdisciplinary Oncology Program, UBC

The metastatic spread of cancer is associated with >90% of cancer-related deaths. Immune modulatory cells are thought to promote metastatic tumor growth, and proteins secreted by poorly oxygenated (hypoxic) tumors can stimulate the accumulation of myeloid-derived suppressor cells (MDSCs) that suppress T-cell mediated immune responses in tissues. Both circulating G-CSF levels and hypoxia in primary tumors correlate with MDSC accumulation in the spleen and lungs of mice bearing metastatic mammary carcinomas.

To address the correlation between tumor hypoxia and MDSC accumulation in metastatic target tissues such as the lungs, we utilized the hypoxia-activated cytotoxin tirapazamine (TPZ) to kill hypoxic tumor cells in vivo. Interestingly, TPZ did not effectively target hypoxic cells in 4T1 tumors, but was directly cytotoxic to MDSCs in the spleen and lungs of tumor-bearing mice despite the fact that MDSCs were not hypoxic in vivo. The magnitude of TPZ-mediated cytotoxicity was comparable to established MDSC targeting chemotherapeutics gemcitabine (GEM) and 5-fluorouracil (5FU). During these studies, we also found that circulating MDSC levels could be used as a reliable, minimally invasive surrogate marker for tissue accumulation of MDSCs.

We also found that MDSC levels in the lungs decreased rapidly upon primary tumor resection, but that MDSCs in the lungs remained significantly elevated compared to naïve animals. Our data indicate that the primary tumor created a long-lasting, MDSC-containing lung environment that supports subsequent metastatic tumor growth. We also used GEM to target residual (post-resection) MDSCs in the lungs to determine the importance of MDSCs in promoting metastatic growth after primary tumor resection. Our data support targeting immune suppressive myeloid cells to treat metastatic breast cancer.

ROLINDA CARTER

Supervisor: Dr. Ed Pryzdial
Session: Basic Sciences

"Anticoagulant rivaroxaban inhibits the novel clot busting function of factor Xa"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Aberrant clots block the flow of blood and contribute to cardiovascular disease, the leading cause of death worldwide. To dissolve these clots, the key physiological clot lysis enzyme, tissue plasminogen activator (tPA) has been developed as a "clot-busting" drug. tPA converts inactive plasminogen into plasmin, the enzyme that directly cleaves and solubilizes the clot. As tPA therapy is associated with significant bleeding, its use is highly restricted because of possible life-threatening hemorrhages. As a result new and safer methods to dissolve clots are needed. Using low concentrations of tPA, our lab has previously demonstrated that two specific cleavages of clotting factor Xa (FXa) by plasmin convert it into a tPA cofactor which enhances clot lysis in vitro. The incorporation of a chloromethyl ketone-type inhibitor into the active site of FXa alters these cleavages and attenuates its tPA cofactor function. This observation suggests that Rivaroxaban (rxbn), a new generation anticoagulant that also binds to the active site of FXa, may have inhibitory or other unexpected effects on clot dissolution. The objective of this study is therefore to address the possibility that rxbn may alter the fragmentation of FXa with consequence to FXa-enhanced clot lysis.

Methods:
In the absence of fibrin, the molecular mesh of the clot, chromogenic assays (S-2251) were used to evaluate the effect of rxbn on FXa enhanced tPA-mediated plasmin generation. These results were correlated to the fragmentation profile of FXa by non-reduced anti-human FX western blots and iodine-125 radiolabelled plasminogen ligand blots. Forming a purified fibrin clot, rxbn was incubated with FXa and the extent of clot formation and dissolution was monitored by Rayleigh light scattering. Samples taken during the course of the experiment were analyzed by western and ligand blots. The cleavage of FXa was evaluated by western blot in normal plasma, plasma obtained from a patient taking rxbn, and normal plasma spiked with rxbn (340nM and 3.4uM). Clot lysis analyses were also carried out using the above mentioned plasmas.

Results:
Rxbn reduced the enhancement of tPA-mediated plasmin generation by FXa. It also inhibited the FXa-mediated enhancement of fibrinogen clot lysis by delaying FXa cleavage and reducing plasminogen binding which is required for plasmin generation. In plasma, rxbn altered the fragmentation of FXa. Plasma clot lysis was enhanced by rxbn in the absence of added FXa and in plasma from a patient taking rxbn. This enhancement was however lost in the presence of pre-formed FXa in complex with rxbn.

Conclusions:
This study shows that rxbn alters the fragmentation profile of FXa, consequently reducing the enhancement of tPA-mediated plasmin generation and inhibiting fibrinogen clot lysis. Interestingly, while rxbn alone had no effect on fibrinogen clot lysis, it enhanced plasma clot lysis, which requires further investigation. This study may therefore highlight unforeseen effects of rxbn on clot lysis. This is of importance as rxbn is approved for the prevention of deep vein thrombosis and one-third of patients with this condition develop post-thrombotic syndrome, a condition with possible links to ineffective or delayed clot lysis.

WAI HANG (TOM) CHENG

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Biomechanical and functional characterization of CHIMERA: A novel closed-head impact model of engineered rotational acceleration"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Traumatic brain injury (TBI) is a leading cause of death and disabilities. A major challenge in TBI research is that many common experimental models do not faithfully replicate the biomechanical aspects of TBI in real-life. To address this issue, we have developed a novel rodent TBI model with high precision, reliability and translatability, called CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration). It is distinct from existing models in that it delivers precise impact to the intact head in a non-surgical procedure, and allows unrestrained head movement. In this study we characterized the biomechanical and acute functional outcomes of repetitive TBI using CHIMERA.

Methods:
Two TBI at 0.5J impact energy were induced to adult C57Bl/6 mice at 24 h apart. Head kinematics were assessed using high-speed videography (5000 fps). Post-injury neurological outcomes, motor function, and anxiety-like behavior were assessed by loss of righting reflex duration (immediately post-injury), neurological severity score (1h, 24h, 48h), falling latency from accelerating Rotarod (24h, 48h), and open-field thigmotaxis (24h), respectively.

Results:
Head kinematic analysis showed a peak linear displacement of 49.6 ± 3.5 mm, and a peak angular deflection of 2.6 ± 0.28 rad. Peak linear and angular velocities were 6.6 ± 0.8 m/s and 305.8 ± 73.7 rad/s, respectively. The head experienced peak linear and angular accelerations of 385.1 ± 52 g and 253.6 ± 69.0 krad/s2, respectively. Injured mice showed significantly prolonged loss of righting reflex, displayed neurological and motor deficits, and anxiety-like behavior.

Conclusions:
CHIMERA is a simple and reliable model of TBI that offers integration of biomechanics and functional assessment.

ERIC DENG

Supervisor: Dr. Honglin Luo
Session: Basic Sciences

"Disrupted growth factor-bound 2-associated-binding protein 1 signaling in coxsackievirus-induced myocarditis"

AUTHORS:
Haoyu Deng[1], Gabriel Fung[1], Paulina Piesik[1], Jingchun Zhang[1], Junyan Shi[1], Honglin Luo[1*]

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Background/objectives:
Background: Viral myocarditis, an inflammatory heart disease induced by virus infection, is the major cause of the sudden death in adolescents and young adults. Epidemiological study reported the long-term mortality of viral myocarditis and its sequelae, dilated cardiomyopathy (DCM), was up to 19.2%. Coxsackievirus B3 (CVB3), an enterovirus in the Picornavirus family, is regarded as one of the most prevalent virus causing myocarditis. It encodes protease 2A and 3C (enteroviral proteases) which induce the pathogenesis of cardiomyocytes by processing the viral polyprotein and cleaving essential host cellular proteins. Growth factor-bound 2 (GRB2)-associated-binding protein 1 (Gab1) is a scaffolding adaptor protein that serves as a platform for the assembly of signalling proteins and plays an important role in signal transduction mediated by various growth factors and cytokines. Mainly, it is responsible for activation of a series of pathways which are crucial to cell apoptosis, proliferation, migration and differentiation. Dysfunction of Gab1 has been shown to cause physiological abnormality in different organs especially heart.
Objective: CVB3 infection causes dysregulation of signalling of Gab1 contributing to the pathogenesis of viral myocarditis and its sequela DCM.

Methods:
In order to determine the potential cleavage events induced by virus, CVB3 with different virus titers (10MOI/100MOI) was used to infect HeLa cells and mouse cardiomyocytes (HL-1 cells) respectively. Western blot was utilized to determine the protein levels of Gab1 in both HeLa cells and HL-1 cells and their potential cleavage fragments. Moreover, qPCR was used to examine the mRNA levels of Gab1 in HL-1 cells and confocal microscopy was utilized to detect the localization of Gab1 in HeLa cells.

Results:
Western blot results demonstrated that protein levels of Gab1 were reduced following CVB3 infection of HL-1 cells and HeLa cells. qPCR results showed that mRNA levels of Gab1 were increased, suggesting a compensatory upregulation of gene expression after protein levels were decreased. Interestingly, we detected two potential cleavage fragments (i.e. 75kDa and 50kDa) of Gab1 in CVB3-infected HeLa cells using an anti-C-terminal Gab1 antibody. Cleavage of Gab1 was verified using a Flag-tagged exogenous Gab1. Treatment of CVB3-infected HeLa cells with z-VAD-FMK didn't eliminate the production of the cleavage fragment, indicating a caspase-independent cleavage event. Two putative cleavage sites by viral protease 2A at G175 and G379 were identified by using a computer server. These two cleavage sites were confirmed using a Gab1-G175E&G379E mutant which was demonstrated to be uncleavable after CVB3 infection. Furthermore, translocation of Gab1 to the cellular membranes was observed in CVB3-infected HeLa cells.

Conclusions:
Degradation of Gab1 caused by CVB3-induced cleavage events may disrupt Gab1 signalling and further contribute to the development and progression of coxsackieviral myocarditis.

GABRIEL FUNG

Supervisor: Dr. Honglin Luo
Session: Basic Sciences

"Coxsackieviral infection causes cytoplasmic translocation and cleavage of TAR DNA binding protein-43"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Coxsackievirus B3 (CVB3) is a non-enveloped, positive single-stranded enterovirus that infects the heart, pleura, pancreas and liver. CVB3 infection can cause myocarditis, and its sequelae, dilated cardiomyopathy (DCM). In North America, DCM accounts for approximately 20% of heart failure and sudden death in children and young adults. Recent findings have shown similar molecular pathologies between cardiomyopathies and neurodegenerative diseases. Cytoplasmic TAR DNA binding protein-43 (TDP-43)-aggregates are a common biomarker of neurodegenerative diseases such as frontotemporal dementia and amyotrophic lateral sclerosis. TDP-43 plays a role in transcriptional and translational regulation during stress and is critical for regular mRNA and protein dynamics in the brain. However, the significance of TDP-43 in CVB3-induced cardiomyopathy remains unstudied. Therefore, we **Hypothesize that CVB3-infection induces formation of cytoplasmic TDP-43-aggregates in viral-induced cardiomyopathy**.

Methods:
HeLa cells were used to express a GFP-tagged TDP-43 and CVB3-infected at an MOI of 10. GFP fluorescence was monitored to observe TDP-43 localization/aggregation by confocal microscopy. To explore the potential mechanism by which CVB3 regulates TDP-43 localization, we examined protein expression of TDP-43 in CVB3-infected cells using immunoblot techniques. 50uM of either Alln or Z-VAD-FMK was used to inhibit calpain and caspase activity respectively. Finally, *in-vitro* cleavage assay was performed using purified viral 3C protease incubated in HeLa cell lysates.

Results:
Confocal imaging showed redistribution of nuclear TDP-43 to cytoplasmic aggregates under CVB3 infection. Furthermore, we found that formation of TDP-43 aggregates are dependent on viral protease 2A activity. We also found that viral protease 3C actively cleaves TDP-43 at late time points of infection, where cleavage is independent of host protease caspase and calpain.

Conclusions:
We conclude that TDP-43 pathologies in CVB3-induced cardiomyopathy may be similar to those previously found in neurodegenerative diseases and may reveal an undiscovered virus-host interaction to cause viral-cardiomyopathy.

The research is supported in part by Canadian Institute of Health Research

PAUL HANSON

Supervisor: Dr. Decheng Yang
Session: Basic Sciences

"Cleavage of death associated protein 5 by coxsackievirus B3 protease 2A causes its nuclear translocation and affects internal ribosomal entry site containing gene translation"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Coxsackievirus B3 (CVB3) is a primary causative agent of viral myocarditis which may progress to dilated cardiomyopathy (DCM), a leading cause of unexpected death in children and young adults. The CVB3 genome is a positive, single-stranded RNA that is translated using an internal ribosome entry site (IRES)-mediated mechanism, as opposed to cap-dependent translation. Death associated protein 5 (DAP5) is an internal ribosomal entry site (IRES) specific translation initiation factor, utilized by the cell in conditions of stress and apoptosis. We previously showed that DAP5 is cleaved during CVB3 infection both in vitro and in vivo. We hypothesize viral protease cleavage of DAP5 during CVB3 infection will alter its cellular distribution and effect translation initiation of IRES-containing mRNAs.

Methods:
2A and 3C genes were cloned into IRES containing vectors and co-transfected into HeLa cells with FLAG tagged WT DAP5. A point mutation at the site of cleavage of DAP5 was generated and transfected into HeLa cells prior to CVB3 infection. N- and C-terminal cleavage products of DAP5 tagged with FLAG or HA, respectively, were cloned into expression vectors to study the cellular localization and function. HeLa cells were transfected with Flag-tagged full-length DAP5, Flag-tagged N-terminal, or HA-tagged C-terminal gene constructs. Nuclear and cytoplasmic proteins were isolated from cell lysates. The proteins were analyzed by western blot or visualized using confocal microscopy to detect ALEXA 594 or 488 secondary antibodies. Bicistronic luciferase reporter plasmid containing the IRES region of Bcl-2 were co-transfected with WT DAP5, N-terminal or C-terminal DAP5 and subjected to luciferase assay to determine their role in IRES-mediated translation.

Results:
We identified viral protease 2A as being responsible for DAP5 cleavage at G434 during CVB3 infection. Upon cleavage, the N-terminal product could translocate to the nucleus, whereas the C-terminal remained localized to the cytoplasm. The N-terminal cleavage product of DAP5 retains translational capability of IRES containing genes, at a reduced level compared to WT. The C-terminal cleavage product is not capable of participating in IRES mediated translation.

Conclusions:
This is the first report to reveal the protease responsible and cleavage site of DAP5 during CVB3 infection. Our functional and mechanistic studies of DAP5 cleavage products reveal they play a role in regulating viral and host gene expression. This study may identify key components necessary for facilitating viral pathogenesis and thus provide novel pharmaceutical targets for viral myocarditis.

ADAM HERMAN

Supervisor: Dr. M Seidman (project mentor) / M Allard (rotation supervisor) / A Krahn (thesis advisor)
Session: Clinical Sciences

"SVC laceration rates in women during laser-assisted lead removal"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Cardiovascular disease remains a major concern in Canada, with approximately 29% of all deaths in Canada attributable to cardiovascular disease in 2008. Many deaths, and even more decreased quality of life, can be attributed to cardiac arrhythmias. These heart rhythm disturbances are increasingly treated with medical devices such as pacemakers and implanted cardiac defibrillators (ICDs). Unfortunately, this increased use of devices carries with it an increased risk of complications in the form of device malfunction and infection, requiring device removal. Lead extraction is often limited by growth of fibrous tissue around the lead. A common strategy to address this uses pulsed ultraviolet laser to burn away the fibrotic tissue. Lasers have been shown to allow for complete lead removal in 94% of patients, compared to only 64% in non-laser procedures. Laser-assisted lead removal, however, also causes a dramatic increase in the rate of superior vena cava (SVC) laceration and rupture. Data from the PLEXES trial, and unpublished data from St. Paul's Hospital in Vancouver, British Columbia, have shown that women are at particular risk for SVC laceration during laser-assisted lead removal, but the reason for this increased risk is currently unknown.
Hypothesis: Women have thinner SVCs than men, resulting in an increase likelihood of SVC lacerations during laser assisted ICD lead removal.

Methods:
Post-mortem tissue from men and women who did not have any previous cardiovascular procedures will be obtained through the Cardiovascular Tissue Registry of the UBC James Hogg Research Centre. The anterior wall of the SVC will be marked with ink and sections will be embedded as per routine formalin fixed paraffin embedded histology procedures. Sections stained with hematoxylin & eosin (H&E), Masson's trichrome, and Movat's pentachrome will be evaluated. Wall thicknesses will be determined using digital image analysis.

Results:
Methods have been validated as being able to robustly characterized SVC wall thickness with a precision of better than +/- 5 microns. Adequate specimens, however, required processing differing from routine material collection in the Registry, and as such material will be collected prospectively from autopsies with research consent signed by the next of kin. The Registry is empowered to collect material from all UBC affiliated sites, and as such it is anticipated that adequate material can be collected over the next 18-24 months. Power calculations estimate that 10-20 samples from each gender will be required to identify the anticipated differences in wall thickness.

Conclusions:
Regardless of whether the increased complication rate in women during laser-assisted ICD lead extraction is found to be due to wall thickness or not, these data will help guide procedures to improve the safety of the laser-assisted lead extraction procedure.

MANU THOMAS KALATHOTTUKAREN

Supervisor: Dr. Jayachandran N. Kizhakkedathu
Session: Basic Sciences

"Understanding the influence of a novel heparin reversal agent on fibrinogen, fibrin polymerization and plasma coagulation "

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Anticoagulation therapy with unfractionated heparin (UFH), low molecular weight heparins (LMWHs) and fondaparinux play a pivotal role in the treatment of thrombosis. The risk of life-threatening bleeding in anticoagulated patients is a major concern. Therefore, excess circulatory anticoagulant level must be controlled by administering an ideal antidote. Protamine sulphate (PS), a cationic peptide is the only clinically approved antidote for UFH. The inability of PS to completely reverse anticoagulation effect of LMWHs and fondaparinux is due to its low binding affinity to these anticoagulants. However, PS exhibit electrostatic interaction with major blood coagulation proteins such as fibrinogen leading to cardiovascular adverse effects. Recently we discovered a novel synthetic multivalent macromolecular universal antidote which can neutralize the anticoagulation actions of all the parenteral clinical anticoagulants. The aim of the current study is to understand the interaction of the antidote with fibrinogen, blood coagulation system and its influence on blood clotting.

Methods:
We designed the polymeric antidote by conjugating tertiary amine based heparin binding groups on a dendritic hyperbranched polyglycerol scaffold and capping it with methoxy polyethylene glycol (mPEG) chains. The cationic heparin binding groups interact with crucial negatively charged groups on heparin based anticoagulants to reverse the anticoagulation action. The length and density of mPEG chains are optimized to prevent non-specific interaction of the antidote with plasma proteins and blood cells. To study the impact of these cationic polymeric antidotes on human plasma coagulation, we performed the recalcification and tissue factor initiated turbidimetric plasma clotting assays. We then investigated the influence of the antidote on fibrinogen by performing fibrin polymerization assay and analyzing the changes in intrinsic protein fluorescence of fibrinogen upon incubation with the antidote. Neutralization of heparin anticoagulation was studied by ex vivo chromogenic factor Xa activity assay in human plasma.

Results:
The turbidimetric plasma clotting assays with the antidote did not significantly affect the clotting parameters such as lag time, maximum absorbance and clotting rate compared to PS. We observed no influence of the polymeric antidote on fibrin polymerization over a broad range of concentrations (50 μg/mL to 1 mg/mL). On the other hand, PS interacted with fibrinogen causing precipitation and aggregation. In addition, incubation of the antidote with fibrinogen did not quench the intrinsic protein fluorescence of fibrinogen. At 100 μg/mL concentration the antidote reversed anti-factor Xa activity of all clinically available parenteral anticoagulants demonstrating its potential.

Conclusions:
Studies confirm that unlike protamine sulphate our heparin reversal agent has negligible impact on fibrinogen, fibrin polymerization and human plasma coagulation suggesting its non-toxic nature and potential clinical utility in the treatment of anticoagulant related bleeding and in cardiothoracic surgery. Universality of the neutralization activity of antidote is demonstrated. Our findings will help us to further refine the antidote design for improving efficacy. Detailed investigation on the ultrastructure of the plasma clots are in progress.

ROBERT KRIDEL

Supervisor: Dr. Randy Gascoyne
Session: Clinical Sciences

"CD23 as a marker of tumour heterogeneity and outcome in follicular lymphoma "

AUTHORS:
Robert Kridel[1], Merrill Boyle[1], Raymond Lim[1], Ayesha Vawda[1], Christoffer Hother[1], Daisuke Ennishi[1], Anja Mottok[1], Fong Chun-Chan[1], Susana Ben-Neriah[1], Stacy Hung[1], King Tan[1], Alden Moccia[1], Graham W. Slack[1], Joseph M. Connors[1], Laurie H. Sehn[1], Christian Steidl[1], Randy D. Gascoyne[1]

AFFILIATIONS:
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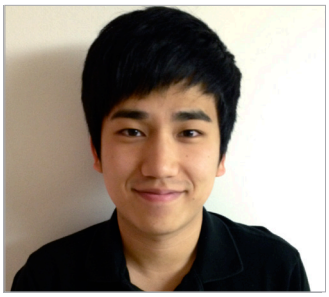
Background/objectives:
Follicular lymphoma (FL) is the most frequent indolent lymphoma and is clinically characterized by highly variable patient outcomes. We hypothesized that prognosis can be predicted from the gene expression profile obtained from formalin-fixed and paraffin-embedded tissue.

Methods:
We generated gene-expression profiles from 185 pre treatment FL samples using the Illumina Whole-Genome DASL platform. The association of gene expression with outcome was tested using univariate Cox regression. We stained a tissue microarray (TMA) matching the discovery cohort by immunohistochemistry (IHC) for CD23 (FCER2). We then calculated transcriptome-wide gene expression correlations with FCER2 and analyzed pathway enrichment within significantly correlated genes using the Reactome FI plugin in Cytoscape. Lastly, we performed phospho-flow on primary patient samples to compare B-cell receptor (BCR) signalling in CD23+ versus CD23- tumour cells.

Results:
A total of 746 genes were associated with survival at a p-value cut-off of 0.05. We chose to focus on FCER2/CD23 as this marker had previously been associated with outcome in FL (Olteanu et al, Am J Clin Path, 2011) and is used in routine clinical flow cytometry panels. Staining of a TMA using IHC revealed substantial heterogeneity between patients (32.3% of cases positive/67.7% negative) and within patients (varying staining intensities between the follicular and interfollicular compartments). Using pathway enrichment analysis, we discovered that genes involved in BCR signalling, antigen processing/presentation and CXCR4-mediated signalling were significantly enriched within FCER2-correlated genes, suggesting that CD23 identifies a subset of biologically distinct FLs. Phospho-flow on 4 primary FL samples further showed that CD23+ cells have a higher response to BCR stimulation than CD23- cells as assessed by pAKT (average fold increase in median fluorescence 3.23 vs 1.91, respectively; ratio-paired t-test 0.015).

Conclusions:
We re-discovered CD23 as a marker of outcome and disease heterogeneity in FL. We further correlated CD23 expression with BCR signalling by gene expression profiling and phospho-flow. CD23 could potentially serve as a biomarker to guide patient management. More specifically, CD23 could represent a predictive marker of response to BCR inhibitors that are under active investigation in a variety of B-cell malignancies.



FRANK LEE

Supervisor: Dr. Edward Pryzdial
Session: Basic Sciences

"Modified clotting factor Xa: dissecting a novel "*clot-buster*"

AUTHORS:
Frank M.H. Lee[1,2,3], Kimberley Talbot[1,4], Scott Meixner[1,4], and Edward L.G. Pryzdial[1,3,4]

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Tissue plasminogen activator (tPA), the physiological initiator of fibrinolysis, has been used as a vital "clot-busting" therapeutic for almost two decades. Our lab has implicated activated clotting factor X (FXa) as an additional accelerator of tPA and has found a way to enhance the fibrinolytic activity of FXa towards therapeutic development. A FXa derivative—known as Xa-K—has been developed involving a C-terminal lysine (C-TL) tethered onto the active site of FXa via a 25 angstrom spacer. Our rationale was the spacer would sterically hinder a cleavage that inactivates the clot-dissolving activity of FXa and the C-TL would further enhance fibrinolytic activity because it is part of a tPA binding site. Our goals were to dissect the role of these two biochemical modifications. In light-scattering turbidometric lysis assays, Xa-K significantly enhanced plasma clot lysis. We compared Xa-K with another FXa derivative—Xa-alanine (Xa-A)—that possesses a C-terminal alanine instead of lysine and found no difference *in vitro*. Using thromboelastography, a technology measuring torsional force transmitted through a sensor placed within a rotating sample, Xa-K showed a dose-dependent effect on enhancing plasma clot lysis and the maximal rate of clot lysis. Our data show that amino acid-tethered coagulation proteins are a viable pursuit for novel "clot-busting" agents, working as auxiliary cofactors in fibrinolysis by assisting fibrin in enhancing tPA function.

JACKY LEUNG

Supervisor: Dr. Marianne Sadar
Session: Basic Sciences

"Novel insights into the implication of N-terminal domain of the androgen receptor: The role as a decoy and the mechanism in prostate cancer"

AUTHORS:
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Background/objectives:
Once prostate cancer has progressed to castration-resistant disease, the treatment options are very limited and the life expectancy is only about two years. As a result, there is a need for more effective therapies which target tumour growth during castrate conditions. In the absence of testicular androgens, activation of the androgen receptor (AR) is believed to continue to play a critical role in castration-resistant prostate cancer (CRPC). Notably, the N-terminal domain (NTD) of the AR has been previously shown to play an important role and is an effective decoy molecule for blocking the growth of prostate cancer tumours. Thus, the AR NTD is a novel therapeutic target for potential treatment of prostate cancer. However, the essential residues and potential mechanisms by which decoys inhibit prostate cancer tumour growth have yet to be determined.

Methods:
In this study, we mapped smaller regions of the AR NTD to prevent the growth and progression of prostate cancer, and introduced a decoy AR NTD ($AR_{1\text{-}558}$) and small decoys ($AR_{1\text{-}233}$ and $AR_{234\text{-}391}$) into CRPC cells, using the most effective lentiviral-mediated gene transfer system for gene therapy.

Results:
Lentivirus delivery of the decoys $AR_{1\text{-}558}$, $AR_{1\text{-}233}$ and $AR_{234\text{-}391}$ was successfully demonstrated to delay hormonal progression in castrated hosts, without affecting other tissues in vivo. We provided evidence that this effect is due not to prevention of the nuclear translocation of the AR, but due to decreasing proliferation and increasing apoptosis. Furthermore, decoy molecules were shown to 1) decrease the expression of androgen-regulated genes; 2) block AR interaction with androgen response elements on DNA; and 3) attenuate phosphorylation of AR by intracellular kinases.

Conclusions:
This study combines gene therapy using lentiviral-mediated delivery, with a potential antitumor decoy molecule and involved molecular mechanisms which together may lead to a clinical application addressing an important therapeutic use in prostate cancer.

VINCENT LEUNG

Supervisor: Dr. Jayachandran N. Kizhakkedathu
Session: Basic Sciences

"Investigation of the interaction of complement with hyperbranched polyglycerol grafted red blood cells"

AUTHORS:
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Background/objectives:
Red Blood Cell (RBC) transfusions are the only treatment available for people living with thalassemia and sickle cell anemia. Due to the frequency of transfusions (25 to 50 transfusions/year), more than 30% of these patients develop alloimmunization, where antibodies are produced against antigens of transfused RBCs. In addition, hemolytic transfusion reactions are a risk due to the multitude of blood group antigens on RBC, some of which are highly immunogenic. Our laboratory is investigating the modification of RBC surfaces by grafting hyperbranched polyglycerols (HPGs), a functional and biocompatible polymer. Our aim is to mask the inherent antigenicity of RBCs. We anticipate that many of the antigens on the RBC surface will be camouflaged or protected by HPGs, thereby developing universal red blood cells that demonstrate functionality in vitro, as well as in vivo. However, previous studies have shown that high molecular weight (MW) and higher surface concentrations of HPG is critical to protect all minor antigens on RBCs. These conditions result in activation of the complement system, consequently lysing the HPG modified RBCs. This study investigates which pathway of complement is involved, and the influence of graft polymer properties on complement activation.

Methods:
Different concentrations and MW HPGs were grafted to RBCs and incubated with pooled human serum for complement activity. To assess the complement activation, traditional assays using antibody sensitized sheep erythrocytes, as well as rabbit erythrocytes were used. In addition, ELISAs were used to determine the exact mechanism and pathway of complement activation. Flow cytometry was performed to demonstrate the effectiveness of antigen protection by the different MW and grafting concentrations of HPGs.

Results:
Above certain HPG grafting concentrations, the modified RBCs activate the alternative pathway. Complement proceeds in assays where calcium, a requirement for the classical pathway, is chelated. This suggests that complement activation occurs via the alternative pathway. This is further supported by ELISA studies. The cleaved product of the alternative pathway Factor B, Bb increased in an HPG concentration dependent manner. This increase is not observed for C4a. Low MW HPGs and grafting concentrations below 1.5 mM demonstrate minimal complement activation, as determined by traditional complement assays. In agreement, ELISA data show low concentrations of complement products C3a, Bb, and SC5b - 9. For HPG concentrations above 1.5mM, there is an exponential increase in complement activation and hemolysis. Furthermore, the functionalities on HPG influences complement activation and hemolysis. An increase is observed when there are more reactive binding groups on HPGs.

Conclusions:
HPG on cell surfaces activate complement via the alternative pathway above certain grafting concentrations, below which, no activation is observed. These insights may lead to a novel method for the development of safe universal red blood donor cells. Successful outcome will have implications not only in transfusion, but also in transplantation medicine.

JONATHAN LIM

Supervisor: Dr. Poul Sorensen
Session: Basic Sciences

"Oncogenic transformation with kras or ETV6-NTRK3 (EN) transcriptionally activates a specific response pathway to protect against oxidative stress"

AUTHORS:
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Background/objectives:
Tumors undergo constant pressure to adapt to stress caused by changing intra- and extracellular environments, but the mechanisms of these processes are still largely elusive. Generally, it is known that oncogenic transformation may lead to a gain or a loss of sensitivity toward stress induction. Previously, we showed that transformation with Kras or EN increases cellular sensitivity to nutrient deprivation (Leprivier et al, Cell, 2013). As such, we were also interested in the response of transformed cells to other forms of stress, such as oxidative stress. Interestingly, it was shown that expression of endogenous Kras mutant, Braf mutant and Myc increased the transcription of the master regulator of the antioxidant response, Nrf2, to lower intracellular reactive oxygen species (ROS) under basal conditions (DeNicola et al, Nature, 2011). Based on these data, we hypothesize that Kras or EN protects cells against oxidative stress-induced cell death. Our objective is to delineate a novel signaling pathway and to determine if inhibition of its downstream effector is a tractable therapeutic strategy. This is important because activating mutations in Kras are found in ~90% of pancreatic cancers, ~40% of colorectal cancers, and ~30% of NSCLC. No effective targeted therapies currently exist for patients of this genetic subset. Thus, identification of genes and signaling pathways involved in cell stress response may present novel downstream targets for the treatment of Kras and EN-related cancers, or for sensitization of these tumors to reactive oxygen species (ROS)-inducing chemo/radiation therapy.

Methods:
Mouse NIH3T3 fibroblasts were stably transfected with KrasG12D, EN, or empty vector (MSCV). Intracellular ROS and cell death assays were performed using flow cytometry with DCFH-DA staining or propidium iodide, respectively. To identify the transcripts upregulated in KrasG12D- or EN-transformed cells, we performed Affymetrix GeneChip 1.0ST expression profiling, and qPCR to corroborate microarray data.

Results:
KrasG12D- and EN-transformed NIH3T3 cells are significantly more resistant to 16 hrs treatment with H_2O_2, as compared to MSCV control cells. Intracellular ROS levels are at least 2-fold higher in MSCV cells compared to KrasG12D or EN cells following 6 hrs treatment with 200 μM H_2O_2. Whole transcriptome microarray data reveal a candidate gene involved in intracellular glutathione regulation that is differentially upregulated in H_2O_2 - treated KrasG12D- and EN-transformed NIH3T3 cells compared to MSCV cells.

Conclusions:
We demonstrate that stable expression of KrasG12D and EN protects NIH3T3 from H_2O_2, and that this is consistent with intracellular ROS levels. Whole transcriptome analysis suggests that oncogenic transformation with Kras or EN transcriptionally activates a specific response pathway to increase intracellular glutathione and protect against H_2O_2 - induced cell death. We speculate that ablation of this pathway has potential as a therapeutic strategy.

YAN MEI

Supervisor: Dr. Jayachandran N. Kizhakkedathu
Session: Basic Sciences

"Investigation on the design and development of novel antithrombotic and anti-adhesion coatings for cardiovascular applications"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Cardiovascular diseases account for an estimated 17 million deaths annually around the world. Therefore, the demand for improved cardiovascular devices is constantly growing. When a device is implanted into the body, a cascade of events is initiated, including protein adsorption, platelet adhesion and activation, and cellular attachment to the implant surface. These events initiate coagulation cascade that may lead to the thrombus formation on the device surface. In addition, microorganisms may grow on the device surface and form biofilms as implant surface could provide an initial attachment site for bacteria. Hence, developing antithrombotic and anti-adhesion coatings to control the interfacial interactions is of particular importance to sustain their functionality and life-time of cardiovascular devices within the body, thereby reducing the mortality rate and medical costs. Functional coatings based on polymer brushes have gained tremendous attention recently due to their excellent biocompatibility and cell-adhesion resistance. However, the composition of polymer brush exhibit the optimal antithrombotic and anti-adhesion/bacterial performance is still unknown. The current screening studies will help us in identifying the optimal coating and a general strategy to reduce the thrombus formation and bacterial adhesion on cardiovascular devices.

Methods:
Polypropylene film was used as a model platform to construct antithrombotic and anti-adhesion coating.The protein interaction on various coatings was determined by measuring the absorption of fluorescently labeled bovine serum albumin and fibrinogen. The platelet adhesion on the surface in platelet rich plasma was determined using scanning electron microscopy. Fluorescently labeled anti-C3b antibody was used to determine the complement activation on the surface as determined by fluorescent microscopy analysis. The adhesion of *Pseudomonas aeruginosa* on brush coatings was assessed by measuring the number of adhered bacteria by luminescence measurements.

Results:
Well-defined functional polyacrylamide brushes with different thicknesses were prepared on polypropylene films by aqueous surface-initiated atom transfer radical polymerization. Protein adsorption on brush coating showed a correlation between protein adsorption and film thickness: all polymer brushes exhibit highest protein resistance at medium grafting thickness. The poly(N,N-dimethylacrylamide) (PDMA) brushes showed better protein resistance compared to other polymer brushes, and the optimized coating based on PDMA brush reduced 95% of BSA adsorption and 80% of fibrinogen adsorption compared to the original surface. The PDMA coating with highest grafting thickness prevented platelet adhesion. Complement activation results showed that the degree of activation mainly depends on the brush composition, primarily determined by the functionalities present on the polymer chains. Bacteria adhesion results showed that the PDMA coating at medium grafting thickness exhibited superior anti-adhesion performance against Pseudomonas aeruginosa, preventing 96% of initial bacterial adhesion compared to the control surface.

Conclusions:
Our findings show that the polymer chemistry, functionality of the polymers and the thickness of the coating influence coating's performance in preventing protein adsorption, platelet adhesion, complement activation and bacterial adhesion. Based on the current results, PDMA coating with optimized thickness presents the best non-fouling character.

DHANANJAY NAMJOSHI

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Neuropathological and biochemical assessment of CHIMERA: a novel closed-head impact model of engineered rotational acceleration"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Traumatic brain injury (TBI) is a leading cause of death and disabilities. About 50,000 Canadians sustain TBI every year. Despite promising outcomes from many preclinical studies, clinical studies have failed to identify effective pharmacological therapies for TBI, suggesting that the translational potential of preclinical models requires improvement. To address the challenge of generating a simple and reliable model of rodent TBI, we have developed a novel neurotrauma model called CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) that fully integrates biomechanical, behavioral, and neuropathological analyses. CHIMERA is distinct from existing rodent neurotrauma models in that it uses a completely non-surgical procedure to precisely deliver impacts of defined dynamic characteristics to intact animal head while allowing unconstrained head movement. In this study we characterized the acute neuropathological and biochemical outcomes of repeated TBI (rTBI) in mice using CHIMERA.

Methods:
Adult, male wild-type mice were subjected to two closed-head impacts spaced at 24 h using CHIMERA. For this, the animal was secured in the supine position on the CHIMERA device and the head was struck with a pneumatically-driven piston. The piston velocity and impact energy were precisely controlled by adjusting the air pressure. Microglial response was assessed by Iba-1 immunohistochemistry while axonal injury was assessed by silver staining at 48 h post-rTBI. Protein levels of TNF-alpha and IL-1beta were measured in half brains collected at 6, 12 and 48 h post-rTBI using ELISA. Phosphorylation levels of endogenous tau were assessed in half brains collected at 6, 12 and 48 h post-rTBI using three antibodies directed against different phosphorylation sites, namely RZ3 (pThr231), PHF1 (pSer396 and pSer404) and CP13 (pSer202). Total murine tau levels were determined by the pan tau antibody DA9.

Results:
Injured brains showed significant widespread microglial activation in white matter areas including olfactory nerve layer (ON), corpus callosum (CC), optic tracts (OT) and brachium superior colliculus (BSC) at 48 h post-rTBI. Microglia in the ON, CC and BSC of injured animals were predominantly hypertrophic to bushy with primary branches only, whereas those in OT showed amoeboid morphology characteristic of activated microglia. Silver staining revealed axonal injury, as indicated by intense punctate and fiber-associated argyrophilic structures in white matter including ON, CC, OT and spetofimbrial nucleus at 48 h post-rTBI. High-magnification silver-stained images revealed numerous axonal varicosities, which is a characteristic histological feature of human axonal pathology. Protein levels of TNF-alpha and IL-1beta showed ~ 1.7- and 2-fold increase, respectively at 48 h following rTBI. Immunoblot analysis showed significantly increased (~1.5 to 3.5-fold) tau phosphorylation at all probed epitopes, peaking at 12 h following rTBI compared to sham brains. The change in tau phosphorylation was due to a significant increase in ratio of phosphorylated tau:total tau, but not as a result of change in total tau level.

Conclusions:
CHIMERA is a simple and reliable model of murine TBI that replicates several aspects of human TBI such as neuroinflammation, DAI as well as tau hyperphosphorylation.

JON OBST

Supervisor: Dr. Marianne Sadar
Session: Basic Sciences

"Chronic exposure to EPI-002 may select for resistant clones in prostate cancer"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
In North American men, prostate cancer is the most commonly diagnosed form of malignancy, and the second leading cause of all cancer-related deaths. The androgen receptor (AR) continues to be a critical therapeutic target in modern therapies. Regrettably, 20%-40% of patients will eventually develop lethal, castration-resistant prostate cancer (CRPC) wherein the AR maintains signalling potential despite castrate levels of its androgen ligand [1-3]. It is thought that resistance to current therapies develop due to exogenous androgen synthesis, amplification of the AR gene and generation of constitutively active AR splice variants which lack the ligand binding domain (LBD) [1-3]. Our group has identified a new class of molecules (EPI) which inhibit the AR by covalently binding to the N-terminal domain (NTD). The NTD contains the activation function-1 region which is essential for interacting with transcriptional machinery. EPI-002 specifically inhibits the AR - both full length and splice variants - and does not inhibit closely related steroid receptors (glucocorticoid, and progesterone) [1,2]. As part of pre-clinical trials, the objective of this study was to determine if resistance to EPI-002 will occur with chronic exposure.

Methods:
LNCaP cells have been cultured for the past year in the presence of either EPI-002 or equal volume DMSO vehicle to generate an EPI resistant, and a long-term passage control sub-line (p>100). Additionally, a parental untreated LNCaP cell line was used in all experiments (p<50). Basal doubling times were determined for each cell line using the AlamarBlue® assay where 5,000 cells were seeded on 96-well plates in their respective media (+/- treatment). Each plate corresponded to a 24 hr time point (0-96 hrs), and contained a standard curve to estimate cell number and reduce inter-plate variability. Doubling time was estimated using an equation recommended by the ATCC: $DT=t*[\ln(2)/\ln(Xe/Xb)]$ [4]. AR and prostate-specific antigen (PSA) protein levels (PSA is dependent upon AR for expression) were determined by Western blot.

Results:
Both the EPI treated and DMSO-control sub-lines exhibited growth rates which were the same as (AlamarBlue®) or faster than (validation) the parental line, indicating resistance has developed in the EPI-002 treated sub-line. Pictures taken at each time point correlate with data generated from the AlamarBlue® experiments. A potential reason for observed variability is differences in cell cycle progression at the time of plating between independent experiments. More replicates are needed to confirm EPI resistance. Importantly, PSA expression was inhibited in the EPI-002 treated cell line only (at all passage numbers), indicating that EPI-002 is still effectively inhibiting the AR.

Conclusions:
Taken together, these data raise the possibility of an AR independent mechanism of resistance developing in response to chronic EPI-002 treatment. Identifying what is driving resistance is of the upmost importance, as current therapies are insufficient for curing CRPC, and many rely heavily upon AR inhibition.

LINNETTE OCARIZA

Supervisor: Dr. Ed Conway
Session: Basic Sciences

"Polyphosphate as a novel therapeutic for age-related macular degeneration"

AUTHORS:
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Background/objectives:
Age-Related Macular Degeneration (AMD) is the leading cause of blindness for people over the age of 50 in the developed world. AMD exists either in the `dry' or `wet' form. `Wet' AMD is characterized by choroidal neovascularization (CNV). Treatments are available and target vascular endothelial growth factors. However, this form accounts for only 10% of all AMD cases. The other 90% is classified as `dry' AMD. This form is characterized by atrophy of the retinal pigmented epithelial (RPE) cells due to accumulation of drusen - a build-up of cellular by-products, inflammatory proteins, and notably, complement proteins. There are currently no treatment options available for `dry' AMD. Although the causes of drusen formation and AMD progression are not completely understood, there is plenty of evidence pointing towards unregulated complement system activation as a major contributor. The molecule polyphosphate (polyP) has recently been discovered as a down-regulator of the complement system, acting on the terminal pathway which is implicated in AMD pathogenesis. PolyP's suppressive effects are both size- and concentration-dependent. This project aims to evaluate the mechanisms and potential utility of polyP as a therapeutic agent against `dry' and/or `wet' AMD. Our hypothesis is that PolyP will have a size- and concentration-dependent protective effect on in vitro and *in vivo* models of AMD.

Methods:
For the *in vitro* studies, RPE cell-lines will be exposed to three different chemical stressors known to lead to AMD-associated damage. The cells will then be treated with varying lengths and concentrations of polyP. To assess the protective properties of polyP, cell viability assays will be conducted, in addition to immunofluorescence and ELISA's to measure molecular markers for inflammation, neovascularization, and complement activation. For the *in vivo* studies, polyP will be intravitreously injected into rat or mouse models of laser-induced choroidal neovascularization (CNV). Immunofluorescence will be used to assess the retinal damage and complement activation.

Results:
We expect that the administration of polyP on stress-induced RPE cells should result in a decrease in cell-death as well as a decrease of molecular markers for inflammation, neovascularization, and complement activation. Similarly, intravitreous injection of polyP in the *in vivo* AMD model should result in decreased fluorescence intensity correlating to retinal damage and complement activation, compared to a positive control. Preliminary data already demonstrate a marked decrease in complement activation markers upon administration of polyP after laser-induction of CNV in the *in vivo* model.

Conclusions:
The results should show that polyP significantly reduces AMD-associated damage caused by known stressors. This is strengthened by our preliminary *in vivo* results. However, the optimal concentration and size of polyP will likely vary for each stressor. Alternatively, polyP may also have off-target effects not involving the complement system. In either case, with the information gathered from this project, not only will a potential novel therapeutic emerge, but it will also further elucidate AMD pathogenesis.

YE QIU

Supervisor: Dr. Decheng Yang
Session: Basic Sciences

"Coxsackievirus B3 Infection induces expression of the heat shock protein Hsp70 to favor its own replication"

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Background/objectives:
Coxsackievirus B3 (CVB3) is one of the predominant pathogens leading to viral myocarditis, an inflammatory disease of the myocardium. During infection, CVB3 exploits the host cellular proteins, such as chaperone proteins, to benefit its own infection cycles. **Heat Shock 70 kDa Protein 1A (Hsp70)** is a chaperone protein induced by various cellular stresses. Induction of Hsp70 was observed in various heart diseases, including viral myocarditis and dilated cardiomyopathy (DCM), a serious end stage of viral myocarditis. Hsp70 is also associated with various viral infections, such as human immunodeficiency virus and hepatitis C virus. During CVB3 infection, although the cap-dependent translation utilized by most cellular genes is inhibited, Hsp70 can still be translated via a cap-independent way. On the other hand, the change of cytosolic calcium induced by CVB3 is a potential inducer of Hsp70. However, to our knowledge, there are no reports on the correlation between Hsp70 and CVB3 infection. Thus, this study is **aimed** to explore the mechanisms underlying the regulation and role of Hsp70 in CVB3 infection. Our **hypothesis** is that CVB3 infection induces Hsp70 expression via transcription enhancement and induction of Hsp70 expression favors CVB3 replication, resulting in immune infiltration in myocardium to facilitate development of viral myocarditis.

Methods:
HeLa cells, HL-1 cardiomyocytes and A/J mice were infected with CVB3 and the expression of Hsp70 during viral infection was determined by quantitative real-time PCR (qPCR) and Western blot (WB). In HeLa cells, the potential upstream transcriptional regulators of Hsp70, including calcium/calmodulin dependent proteins kinase CaMKII and Hsp70 transcriptional factor HSF-1, were detected by WB and further verified by siRNA-mediated knocking-down. To study the role of Hsp70 in viral replication, Hsp70 was knocked-down by specific siRNAs in CVB3-infected HeLa cells and HL-1 cardiomyocytes, and then viral replication was determined by quantifying viral RNAs and viral proteins using qPCR and Western blot, respectively. Finally, Hsp70 protein secretion, which is critical for immune infiltration, was determined by ELISA in the cell culture.

Results:
We found that both mRNA and protein levels of Hsp70 were increased in cell culture and mouse hearts after CVB3 infection. CaMKII and HSF-1 were activated by phosphorylation during CVB3 infection, corresponding to the up-regulation of Hsp70. Viral genomic RNA and viral proteins were decreased when Hsp70 was knocked down. Increased concentration of Hsp70 was observed in the extracellular medium when the cells were infected by CVB3.

Conclusions:
Our results suggest that CVB3 infection induces the expression of Hsp70 via phosphorylation and activation of CaMKII and HSF-1, which favors CVB3 replication and leads to Hsp70 secretion, a factor associated with immune cell infiltration.

TISSA RAHIM

Supervisor: Dr. Jacqueline Quandt
Session: Basic Sciences

"The use of aryl-hydrocarbon receptor nuclear translocator 2 expression patterns as an indicator of pathogenesis in in vitro models of multiple sclerosis"

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Background/objectives:
Multiple Sclerosis (MS) is the most common neurological disease of young adults in Canada, currently affecting an estimated 100,000 people. MS is a chronic inflammatory and demyelinating neurodegenerative disorder of the central nervous system, characterized by infiltration of inflammatory cells, followed by neuronal damage and loss. Individuals with MS commonly experience devastating symptoms such as loss of balance, impaired vision/speech, extreme fatigue and paralysis. Effective MS treatments that focus on blocking inflammation can be associated with risks such as fever, muscle aches, fatigue, and headaches. For this reason, investigating neuroprotective as well as inflammation-limiting therapeutic targets is crucial. Our lab has become interested in ARNT2 (aryl-hydrocarbon receptor nuclear translocator 2), a transcription factor that functions in neuron survival. In a mouse model of MS, ARNT2 levels reached their lowest points when disability was highest, and rescue of disability with administration of the antioxidant Tempol (4-hydroxy -2,2,6,6- tetramethylpiperidine-N-oxyl) was associated with restored ARNT2 levels. Our objective was to examine the functional relevance of ARNT2 expression patterns in MS and its models; specifically, the ability of inflammatory cells or neuronal stressors to influenced ARNT2 expression. We hypothesized that ARNT2 levels are influenced by inflammatory cells and neuronal stressors.

Methods:
Primary cortical and leukocyte cultures were established or collected from embryonic mouse cortices and spleens, respectively. Cortical cultures were co-cultured with isolated T cells from spleen (using anti-CD3), with potassium chloride, a pharmacological agent known to induce neuronal stress, and with Tempol. Following co-culture, cells were harvested, and western blots were performed for ARNT2.

Results:
Preliminary results showed that spleen-isolated T cells induced altered ARNT2 expression levels in cortical neurons, with an initial peak, followed by a gradual decline across 20 hours, which was associated with reduced cell viability (via visual measurement). Additionally, we found potassium chloride exposure had no significant effect on ARNT2 levels in cortical cultures at a range of 1-8 hours exposure time. Furthermore, this effect was not significantly influenced by Tempol exposure.

Conclusions:
From these preliminary results, we can predict that ARNT2 expression patterns are influenced by the addition of T cells, but are not affected by potassium chloride exposure, in combination with Tempol. The major cause of neurological disability in MS is neuronal damage. Therefore, improving our understanding of ARNT2 and its role in neuronal survival can can aid in identifying novel therapeutic targets to limit disease progression. Furthermore, expression patterns of ARNT2 may serve as a biomarker for MS pathogenesis. Our next steps are to continue characterizing ARNT2 expression in response to immune cells and various cell stressors. Additionally, we will examine the consequences of silencing or overexpressing ARNT2 in primary cortical neuron-enriched cultures and in the mouse model of MS.

DAVID ROWBOTHAM

Supervisor: Dr. Wan Lam
Session: Basic Sciences

"ELF3 is a novel oncogene frequently activated by genetic and epigenetic mechanisms in lung adenocarcinoma"

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Background/objectives:
Lung cancer remains the cause of the most cancer-related deaths each year, with a 5 year survival rate of less than 15%. The predominant type of lung cancer adenocarcinoma (AC). Oncogenes such as EGFR and KRAS are well defined drivers of AC, but in approximately 50% of cases the driver alterations are unknown. ELF3 is an member of the E-Twenty Six (ETS) transcription factor family, and has been reported to be overexpressed in a handful of clinical AC cases and cell lines. A comprehensive analysis of the extent and impact of this overexpression is lacking. The objectives of this study were to determine the frequency of ELF3 disruption in lung AC, identify the genetic causes underlying this disruption, and to characterize the function of ELF3 in lung AC.

Methods:
ELF3 was interrogated in a multidimensional integrative manner in a panel of 83 AC tumors and matched adjacent non-malignant tissues. ELF3 expression was also assessed in The Cancer Genome Atlas (TCGA) database. Stable ELF3 mRNA knock-down AC cell models were used to assess the role of ELF3 in cell viability, proliferation, and tumor growth in vivo. In addition, an ELF3 overexpression model was established in immortalized Human Bronchial Epithelial Cells (HBECs) to assess proliferation in a non-malignant model system. Subcellular localization of ELF3 was determined by western blot and immunofluorescence.

Results:
ELF3 was found to be frequently overexpressed in our cohort (72%) and the TCGA cohort (80%). This upregulation correlated significantly with high frequencies of copy number gain (49%) and hypomethylation (71%), often seen within the same tumor. In fact, 82% of tumors with ELF3 overexpression had concurrent gain and/or hypomethylation of the ELF3 locus. Knock-down of ELF3 in cell models led to significantly reduced cell viability and proliferation, and a similar trend was observed in vivo. Western blot and IF revealed ELF3 to be predominantly located in the nucleus, indicating ELF3 likely behaves through its transcription factor activity. A similar hyperproliferative phenotype was seen in HBEC ELF3 overexpression models.

Conclusions:
The high frequency of ELF3 overexpression (>70%) observed in lung AC is accompanied by frequent DNA-level selection events. The affect of ELF3 on cell proliferation suggests that ELF3 is a novel oncogene in lung AC. Further studies are warranted to determine the mechanism by which ELF3 drives hyperproliferation and potentially other oncogenic functions to define novel drugable targets for this disease.

SARA SABERI

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Longitudinal leukocyte telomere length during pregnancy and post-partum in HIV$^+$ women on combination antiretroviral therapy compared to HIV$^-$ pregnant women "

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Background/objectives:
Women of childbearing age constitute ~50% of HIV+ people worldwide, and 90% of HIV+ children acquire the virus through their mother. Treatment with combination antiretroviral therapy (cART) during pregnancy and delivery can reduce vertical transmission from ~25% to <2%. Both cART and HIV viremia can affect telomerase activity and oxidative stress, potentially modulating leukocyte telomere length (LTL), a marker of aging and predictor of lifespan. The effect of pregnancy, HIV and cART use during pregnancy on LTL is unclear. The aim of this study was to investigate the effect of cART initiation/interruption, and other relevant factors on LTL in pregnancy and post-partum.

Methods:
Relative LTL was assessed at several visits in pregnancy (13-23, 23-31, and 31-40 weeks of gestation) in HIV+ (n=97) and HIV- (n=63). For HIV+ women, LTL was also assessed at delivery and at 6-weeks post-partum. A multivariate linear nested mixed-effects model was applied to investigate differences in LTL between HIV+ and HIV- women, and changes in LTL during pregnancy.

Results:
The median [IQR] ages were similar in the HIV+ and HIV- groups (30 [26-34] vs. 32 [28-35] years, p=0.49). Of the 97 HIV+ women, 25 conceived on cART, while 72 started cART during their pregnancy and 54 continued cART after delivery. LTL were not significantly different at pregnancy visits between HIV+ and HIV- women after controlling for repeat measurements within women in multivariate mixed-effects model (p=0.58). Within HIV+ women, no significant change in LTL was seen upon cART initiation/interruption. LTL measurement in the same women at different times during pregnancy were stable and correlated (intraclass correlation = 0.75). Within HIV+ women, three variables were significantly associated with LTL: CD4 at visit (p=0.006), pVL (p<0.001), and time of visit (pregnancy and delivery vs. post-partum) (p=0.003). Specifically, the estimated model suggests that for every increase in 10 cells/µl of CD4 at visit, the LTL increases by 0.0002 ± 0.0001, and having a detectable pVL is associated with a 0.07 ± 0.04 increase in LTL. In HIV+ women, post-partum LTL is on average 0.12 ± 0.04 (p=0.003) shorter than during pregnancy and delivery.

Conclusions:
Preliminary results suggest that LTL were not significantly different during pregnancy between HIV+ and HIV- women. For HIV+ women, LTL decreased significantly after delivery, regardless of cART continuation or interruption. Due to no significant difference in LTL during pregnancy between HIV+ and HIV- women, the significant decrease in post-partum LTL in HIV+ women may be related to pregnancy rather than HIV/cART. Delivery and post-partum samples were not collected from HIV- women in our cohort; thereof, this study is limited to draw general conclusions with respect to LTL during pregnancy versus post-partum. However, this is the first study examined LTL in the context of either pregnancy or within person cART initiation/interruption, suggesting that LTL increases slowly and steadily as pregnancy progresses, irrespective of HIV/cART.

MEHUL SHARMA

Supervisor: Dr. David Granville
Session: Basic Sciences

"Extracellular Granzyme K mediates endothelial activation through its interaction with protease activated receptors (PAR-1)"

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Background/objectives:
Microbial infections have the potential to cause wide-spread inflammatory immune responses or sepsis. The complex pathology of sepsis prevents its resolution using simple measures, like anti-microbial drugs. The progression of sepsis often leads to widespread tissue hypoxia and eventually organ failure. Previous research has shown a role for endothelial cells in the progression of the disease. Recently the serine protease, Granzyme K (GzK), was detected at significantly higher levels, in serum from patients with sepsis while its inhibitor, inter-alpha inhibitor protein (IaIp), was found to be at considerably lower levels. The increased levels of serine proteases, such as GzK, trigger the cleavage of protease activated receptors (PARs). The activation of PAR leads to downstream effects including cytokine production, cell proliferation, migration and extracellular matrix synthesis. Previous studies show extra-cellular GzK is present in acute lung inflammation and contributes to disease progression by activating PAR-1 and its downstream inflammatory cascade in lung fibroblasts.We hypothesize that extracellular GzK induces endothelial activation through the activation of PARs, contributing to the progression of sepsis.

Methods:
To assess the effects of extracellular GzK on cell proliferation, we treated cultured monolayers of human umbilical vein endothelial cells (HUVECs), with GzK at 1 nM for 24 h. We then conducted an MTS assay and measured absorbance at 570 nm to assess cell proliferation. To study cytokine production, we collected the supernatant of these cultures and screened for MCP-1 using ELISA. We also plan to use immunofluorescence to stain for the Vascular cell adhesion protein-1 (VCAM-1), which facilitates the adherence and migration of inflammatory cells across the endothelial barrier.

Results:
Extracellular GzK administration at a concentration of 1nM is enough to cause an increase in MCP-1 production from HUVECs ($p < 0.05$, $n=6$), and cause cell proliferation ($p<0.05$, $n=6$). This response was diminished when the HUVECs were pre-treated with PAR-1 neutralizing antibody ($p < 0.001$, $n=6$).

Conclusions:
NK cells are shown to play a role in sepsis development. The release of high levels of GzK by these cells in the absence of IaIp production could contribute to the onset and progression of sepsis. We show that extracellular GzK induces pro-inflammatory changes in HUVECs through the cleavage and activation of PAR-1. By exploring the pathways through which GzK has an effect on endothelial activation, we hope to make a strong case for the use of GzK in the diagnosis of sepsis, and GzK inhibitors for sepsis treatment.

JUNYAN SHI

Supervisor: Dr. Honglin Luo
Session: Basic Sciences

"Disrupted selective autophagy in enteroviral-induced myocarditis"

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Background/objectives:
Coxsackievirus B3 (CVB3) is one of the predominant viruses causing viral myocarditis. Myocarditis and its sequela dilated cardiomyopathy (DCM) account for ~20% of heart failure and sudden death in children and youth in North America. Coxsackievirus infection induces an abnormal accumulation of ubiquitin aggregates which are generally believed to be noxious to the cells and play a key role in viral pathogenesis. Autophagy is a protein degradation machinery. Selective autophagy mediated by autophagy adaptor proteins, including sequestosome 1 (SQSTM1/p62) and neighbor of BRCA1 gene 1 protein (NBR1), is an important pathway for disposing of misfolded/ubiquitin conjugates. In this study, we explored the functional importance of SQSTM1/p62 and NBR1 in the disposal of protein aggregates through selective autophagy.

Methods:
We first examined the protein and mRNA levels of SQSTM1/p62 and NBR1 by Western blotting and RT-PCR, respectively. The decrease of the protein levels is due to the cleavage of these proteins by CVB3 protease 2A and 3C as confirmed by *in vitro* cleavage assay. Next, we identified the cleavage sites of these proteins by constructing a series of point mutants. Finally, functional study was performed to explore the binding properties of SQSTM1/p62 cleavage fragments using immunoprecipitaion. Immunostaining and cell fractioning were employed to investigate the cellular distribution and dominant-negative functions of SQSTM1/p62 and NBR1 cleavage fragments.

Results:
We found that SQSTM1/p62 was cleaved at amino acid G241 by protease 2A. NBR1 was cleaved by both proteases 2A and 3C at amino acid G402 and E682, respectively. The cleavage of SQSTM1/p62 resulted in the loss of its function in binding ubiquitin aggregates. Furthermore, we showed that the C-terminal fragments of SQSTM1/p62 and NBR1 exhibited dominant-negative effects against native SQSTM1/p62, probably by competing for LC3 and ubiquitin chain binding. Finally, we demonstrated a positive, mutual regulatory relationship between SQSTM1 and NBR1 during viral infection

Conclusions:
Coxsackievirus infection induces increased accumulation of ubiquitin conjugates through the disruption of selective autophagy mediated by SQSTM1/p62 and NBR1. Understanding the role of selective autophagy in viral myocarditis will provide valuable insights into the mechanisms by which virus utilizes to facilitate its infection and myocardial damage.

TIANQING YANG

Supervisor: Dr. Poul Sorensen
Session: Basic Sciences

"Characterization of hace1 function in oxidative stress by mass spectrometry"

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Background/objectives:
Hace1 is a tumour suppressor identified in our laboratory that is downregulated in many cancers, including various lymphomas, leukemias, and carcinomas. Hace1 is characterized as a HECT family E3 protein-ubiquitin ligase, whose function is to conjugate ubiquitin moieties onto substrate proteins, a modification that may target the substrate protein for proteasomal degradation. Hace1 also has antioxidant properties, as it decreases cellular Reactive Oxygen Species (ROS) levels when exogenous oxidative stress is applied to cells. Currently, Hace1 function is poorly defined, and few interactors and substrates of Hace1 have been identified. Our aims are twofold: 1) to identify interactors of Hace1 that may account for its tumour suppressor activity, and 2) to identify proteins regulated by Hace1 under oxidative stress conditions.

Methods:
To identify Hace1 interaction partners under ambient conditions and oxidative stress, MCF7 cells stably expressing control vector or HA-Hace1 were SILAC (Stable Isotope Labelling by Amino Acids in Cell Culture)-labelled for 7 doublings. Subsequently, they were treated with control media or H2O2, and subject to Affinity Purification - tandem Mass Spectrometry (AP-MS/MS) analysis with anti-HA antibody. To assess Hace1's role in regulating protein synthesis after oxidative stress, MCF7 cells as described above were pulse-labelled in SILAC media for 24 hours, treated with control media or H2O2, and analyzed by LC-MS/MS. To assess Hace's role in regulating protein degradation after oxidative stress, the same MCF7 cells were SILAC-labelled for 7 doublings, and incubated in unlabelled media for 24h. Cells were then treated with control media or H2O2 followed by LC-MS/MS analysis. Relative quantification in all experiments was performed using MaxQuant software.

Results:
AP-MS/MS identified new putative interactors for Hace1, some of which were enriched under oxidative stress compared to ambient conditions. Synthesis and degradation of certain proteins were differentially regulated by Hace1 under oxidative stress compared to ambient conditions.

Discussion and Future Directions:
We will validate candidate Hace1 interactors and to elucidate their role in tumour suppression or the oxidative stress response. We will use AP-MS/MS to examine the ubiquitin-modified proteome under oxidative stress, and compare this to the set of proteins whose degradation are affected by Hace1; we can infer that the overlapping subset represents proteins that are directly targeted to the proteasome by the E3 ubiquitin ligase activity of Hace1. These studies will allow us to pinpoint mechanisms in tumour suppression or the oxidative stress response that revolve around Hace1.

AMAL EL-NAGGAR

Supervisor: Dr. Poul Sorensen
Session: Basic Sciences

"Translational regulation of HIF1α by YB-1 in tumour angiogenesis"

Background/objectives:
Tumour angiogenesis, an essential component of metastasis, is a common feature of malignant neoplasms and is driven by reduced local oxygen tension, or hypoxia. Hypoxia-induced factor 1 alpha (HIF1α) plays a central role in the hypoxia-driven cellular and molecular events that initiate and maintain tumour angiogenesis. One poorly understood aspect of the hypoxic response is the reprogramming of the translational machinery that leads to general inhibition of cap-dependent translation but simultaneous activation of cap-independent translation of certain mRNAs including HIF1α and VEGFA, which mediate angiogenesis to facilitate adaptation of tumours to hypoxia. Y-box binding protein 1 (YB-1) is a DNA/RNA binding protein that contributes to stress associated translational regulation in tumour cells by several mechanisms. First, it globally represses translation of growth-related messages such as cyclins, thus blocking cell proliferation to preserve energy. Second, YB-1 mediates an epithelial-to-mesenchymal transition (EMT) in epithelial tumours by translationally activating the EMT factors, Snail and Twist. However, a link between YB-1 and the hypoxic response has to date not been described.

Methods:
To study potential role of YB-1 in human sarcomagenesis, we used MNNG and MG63 (osteosarcoma), TC32 and TC71 (Ewing sarcoma), and Rh30 and Rh18 (rhabdomyosarcoma) cell lines, and performed transient and stable YB-1 knockdown in each cell line.

Results:
YB-1 was highly expressed across all malignant cell lines tested compared to benign cells. Moreover, we found that YB-1 expression correlates with poor outcome and metastatic disease in Ewing sarcoma. YB-1 siRNA knockdown (kd) significantly reduced migration and invasion of sarcoma cell lines, and this correlated with dramatic reduction of HIF1α protein expression. Moreover, ectopic HIF1α expression restored these parameters in YB-1 kd cells. We then used the mouse renal subcapsular implantation model to xenotransplant human sarcoma cell lines under the renal capsules of NOD/SCID mice to show that YB-1 contributes to tumour cell invasion and metastasis in vivo. Both features were dramatically reduced by YB-1 kd, which correlated with decreased HIF1α expression, but invasion and metastasis in YB-1 kd tumours was rescued by ectopic HIF1α re-expression. Notably, renal subcapsular primary implantation site tumours with YB-1 kd exhibited extensive areas of hemorrhage and necrosis compared to control tumours. This further correlated with reduced HIF1α and VEGFA expression, as well as microvessel density, and therefore highly suggestive of impaired angiogenesis. We therefore tested whether YB-1 might regulate HIF1α expression directly. We found that total HIF1α mRNAs are not increased by YB-1, however its polysomal fractions; the translationally active components, are markedly enriched in polysomes of control compared to YB-1 kd sarcoma cells. This suggests that, like Snail and Twist, YB-1 regulates HIF1α expression at the translational level. YB-1 itself was also induced by hypoxia, and blocking this induction blocked HIF1α protein levels under hypoxia.

Conclusions:
YB-1 plays a crucial role in the adaptive response to hypoxia in sarcomas, and this contributes to sarcoma metastasis. Further, targeting the YB-1-HIF1α axis may represent a novel strategy for the treatment of sarcomas.

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NAGARAJAN (RAJ) KANNAN

Supervisor: Dr. Connie Eaves and Dr. Samuel Aparicio
Session: Basic Sciences

"Human mammary lineage-specific mechanisms of DNA repair"

AUTHORS:
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Background/objectives:
Both endogenous (eg intracellularly produced ROS) and exogenous (eg ionizing radiation) mediators of DNA damage pose a continuous challenge to the maintenance of genomic integrity in normal human mammary cells. In most human breast cancers, this functionality is sufficiently perturbed to induce a state of major genomic instability. Yet very little is known about the mechanisms that maintain DNA integrity in normal mammary cells. These consist of two major lineages that constitute the outer basal and inner luminal layers of the normal mammary gland and include subsets of "lineage-committed" progenitors. We have previously identified oxidative DNA damage control and multiple anti-oxidant mechanisms that give luminal progenitor cells greater resistance to genotoxic stress from H2O2 and X-rays than the progenitors in the basal fraction. The objective of the current study was to identify the nature and function of DNA repair mechanisms that are activated when DNA breaks are induced in these two different human mammary cell subsets.

Methods:
Histologically normal breast cells were obtained from reduction mammoplasties and basal and luminal clonogenic progenitor-enriched fractions isolated by FACS based on their shared positive expression of CD49f and differential expression of EpCAM (final purities of ~20%). Clonogenic activity was assessed in 8-10 day 2D assays containing FBS, irradiated fibroblasts and EGF-containing SF7 medium. The ability of clonogenic cells to repair sublethal damage caused by X-irradiation was tested using split doses of 2+2 Gy at intervals of 0, 5, 30, 120, 240 minutes. To investigate non-homologous DNA end joining (NHEJ) activity in cells, a dual RFP and GFP fluorescent reporter plasmid was created. In either a circular or linearized form, this plasmid directs RFP expression by a PGK promoter but GFP expression by the linear form requires religation of the free ends by NHEJ. Cells were electroporated with this plasmid using an Amaxa nucleofection kit and then cultured for 48-72 hours prior to analysis of RFP and GFP expression by FACS. Untransfected cells and cells transfected with the circularized plasmid served as controls.

Results:
The split-dose X-irradiation experiments performed on mammary cells from 2 different donors showed that basal progenitors displayed less damage (increased survival) with as little as a 30 minute interval between the two doses whereas luminal progenitors showed no such activity for at least 4 hours. Comparison of the NHEJ activity of basal and luminal progenitor cells isolated from 3-5 day cultures of mammary cells showed this activity to be significantly lower in the luminal cells ($p<0.0003$; 35% vs 80% in patient-matched basal cell isolates). Analysis of transcripts from existing global transcriptome datasets generated from similarly purified cells suggest that multiple genes (namely PRKDC/DNA-PK, XRCC6/Ku70, XRCC5/Ku80, DNA Ligases III and IV) in this pathway are significantly downregulated in luminal progenitor cells compared to basal cells.

Conclusions:
These findings suggest that the production of normal human luminal progenitors is accompanied by the acquisition of a DNA quality control state that can reduce DNA damage through enhanced anti-oxidant mechanisms that may compensate for an increased deficiency in the NHEJ mechanism that repairs DNA damage.

LEIGH PARKINSON

Supervisor: Dr. David J. Granville
Session: Basic Sciences

"Granzyme B contributes to extracellular matrix degradation in UV-treated skin"

AUTHORS:
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Background/objectives:
Extracellular matrix (ECM) degradation is a hallmark of many chronic inflammatory diseases that can lead to a loss of function, aging and disease progression. Granzyme B (GzmB), a serine protease that is expressed by a variety of cells, has been shown to accumulate in the extracellular space during chronic inflammation and cleave a number of ECM proteins. Using a model of UV-induced chronic inflammation in the skin, we hypothesized that GzmB contributes to ECM degradation through both direct cleavage of ECM proteins and indirectly through the induction of other proteinases to lead to a phenotype of aged skin.

Methods:
Wild-type and GzmB-knockout (KO) mice were repeatedly exposed to minimal erythemal doses of solar simulated UV-irradiation for up to 20 weeks. Blinded clinical scoring of wrinkle formation was performed by 3 independent assessors, and tissue was collected and fixed for histological and immunohistochemical analyses. Mutli-photon microscopy was employed to assess collagen density and organization. *In-vitro* protein and cell-based inhibition assays were performed to support the *in vivo* findings.

Results:
GzmB expression was significantly increased in wild-type UV-treated skin compared to non-irradiated controls. GzmB deficiency significantly protected against the formation of wrinkles and the loss of dermal collagen density, which was related to the cleavage of decorin, an abundant proteoglycan involved in collagen fibrillogenesis and integrity. Cleavage of decorin by GzmB rendered collagen fibrils more susceptible to attack by matrix metalloproteinase (MMP) 1. GzmB also cleaved fibronectin, and fibronectin fragments have been shown to increase the expression of collagen-degrading MMPs in fibroblasts.

Conclusions:
Collectively, these findings indicate a significant role of GzmB in ECM degradation of UV-treated skin. This work also has implications in many other chronic inflammatory conditions where ECM degradation contributes to the pathogenesis of the disease.

ROBERT JEROME

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Development of an engineered base cerebrovasculature model to study Alzheimer's disease *in vitro*"

AUTHORS:
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Background/objectives:
Alzheimer disease (AD) is the most common cause of cognitive decline in the elderly, and affects more than 40% of people over 85 years of age. In addition to the defining neuropathological hallmarks of neurofibrillary tangles and parenchymal amyloid deposits within brain tissue, over 80% of AD patients also develop cerebrovascular amyloid deposits known as cerebral amyloid angiopathy (CAA). As the presence of cardiovascular and metabolic risk factors including hypertension, type II diabetes and dyslipidemia also increase AD risk, cerebrovascular function is believed to play a critical role in the etiology of AD, likely through regulating the clearance of amyloid-beta (A) peptides from the brain. The cerebrovasculature has two major known mechanisms to facilitate A clearance. First, A interacts with the LDL receptor related protein (LRP) to directly cross the blood brain barrier (BBB). Second, A can be cleared via interstitial fluid drainage pathways that form the equivalent of the lymphatic system in the central nervous system (CNS). However, a critical roadblock to progress in delineating how cerebrovascular function contributes to AD pathogenesis is the lack of model systems that recapitulate the complexity of cerebral blood vessels.

Methods and Results:
We recently engineered a novel model system using primary human endothelial cells (EC) and smooth muscle cells (SMC) cocultured under pulsatile, native-like flow condition that produces a three dimensional (3D) artery equivalent. Histological analyses showed the formation of a dense tissue composed of a tight monolayer of endothelial cells supported by a basement membrane and multiple smooth muscle cell layers. We will in a close future investigate if the engineered model developed CAA when expose to A.

DRAGOS M. VASILESCU

Supervisor: Dr. James C. Hogg
Session: Basic Sciences

"Comprehensive assessment of the human lung by combining stereology based sampling with computed tomography"

AUTHORS:
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Background/objectives:
Multi Detector Computed tomography (MDCT) and micro CT (μCT) have been used to evaluate regional differences in mean linear intercept (Lm) and the total number of terminal bronchioles in human lungs (N Engl J Med 2011;365:1567). This report extends this procedure by implementing a stereology based sampling design.

Methods:
The left lung from two separate organ donors was fully inflated with air (transpulmonary pressure 30cmH20), deflated to 10cmH2O and held at that pressure while rapidly frozen in liquid nitrogen vapor. Each specimen was kept frozen on dry ice while an MDCT scan was performed. Then the specimen was cut into 2cm thick consecutive slices. These slices were kept frozen on dry ice at -800C while photographed. A systematic uniform random design was used to select sampling sites from the photographs. Ten cores of frozen tissue (1,5cm in diameter) removed from the selected sites were fixed with a 1% solution of glutaraldehyde in pure acetone (freezing point -930C) pre-cooled to -800C and fixed overnight at -800C to prevent tissue collapse. They were then warmed to room temperature, critically point dried and imaged using a μCT apparatus (Zeiss, microXCT-400). A stereological assessment of Lm, surface area and volume fraction of the parenchymal components were obtained from 10 regularly spaced μCT images from each core. The number of terminal bronchioles per mL was determined by examining the entire scan of each core.

Results:
The average volume for the two donor left lungs measured by image segmentation of the MDCT lung scans was 3551mL (SD212). Based on the stereological assessment the mean Lm was 339.9μm (SD16.6) with a total surface area of 37.4m2 (SD0.8) per left lung. The mean number of terminal bronchioles per mL was 3.4 (SD1.7) and their total number per left lung was 11947 (SD5500). The volume fractions of parenchymal (par) components were Vv(alveoli/par) = 0.71, Vv(duct/par) = 0.12 and Vv(tissue/par) = 0.17.

Conclusions:
Combination of MDCT with μCT examination of randomly selected samples provides anatomic data comparable to previous reports based on quantitative histology and lung casts with the advantage that it provides 3 dimensional information of the lung anatomy. Companion samples kept frozen at -800C are available for future molecular studies (Genome Med. 2012 4, 67). A disadvantage of our imaging is that the 10 fold lower resolution of μCT compared to electron microscopy yields lower surface area values than EM (Respir Physiol. 1978 Feb;32(2):121-40).

YEMIN WANG

Supervisor: Dr. David Huntsman
Session: Basic Sciences

"The oncogenic role of DICER1 RNase IIIb domain mutations in ovarian sertoli-leydig cell tumors"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
We have recently identified frequent hotspot somatic mutations in RNase IIIb domain (simplified as 'hotspot mutations') of DICER1, an endoribonuclease required for miRNA biogenesis, in ovarian Sertoli-Leydig cell tumors (oSLCTs), a rare class of sex-cord stromal cell tumors that occur in young female. Here, we aim to elucidate how DICER1 hotspot mutations promote the development of oSLCTs.

Methods:
Histological features of oSLCTs were evaluated by pathologists to address the impact of DICER1 hotspot mutation on disease status. Illumina small RNA sequencing and realtime RT-PCR were used to quantitate miRNA expression. Illumina DASL whole trancriptome analysis, Nanostring nCounter system and immunohistochemistry were used to determine gene expression profile. Multiple cell line models are being used to assess the oncogenic potential of DICER1 hotspot mutations.

Results:
A strong correlation was observed between DICER1 hotspot mutation and oSLCTs with retiform pattern, which is often malignant with worse prognosis. The expression of 5p-miRNAs was dramatically reduced in mouse embryonic stem cells expressing ectopic DICER1 hotspot mutations and ovarian SLCTs carrying DICER1 hotspot mutations compared to their counterparts without DICER1 hotspot mutation. A subset of 3p-miRNAs was increased in oSLCTs with DICER1 hotspot mutation. The miRNA production defect was associated with deregulation of genes governing cell fate determination in ovary, such as Aromatase, FOXL2 and FGF9, and genes that are let-7 targets, such as HMGA2, DICER1, Arid3a and Cdc25A. Consistently, knockin of DICER1 hotspot mutation in SVOG3e cells (immortalized human granulosa cells) led to altered expression of genes crucial for ovarian gonadal genesis and cellular transformation. Cells expressing DICER1 hotspot mutation proliferate faster than cells with wildtype DICER1.

Conclusions:
DICER1 hotspot mutations cause systemic loss of 5p-miRNAs and selective upregulation of some 3p-miRNAs due to feedback induction of mutant DICER1. Collectively, DICER1 hotspot mutations may drive pseudo-differentiation of testicular elements in ovary and cause oncogenic transformation.

DAILIN LI

Supervisor:
Session: Clinical Sciences

"Measurement of serum free cortisol in septic shock patients"

AUTHORS:
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Background/objectives:
It is important to identify septic patients with relative adrenal insufficiency who may benefit from corticosteroid therapy. Free cortisol is the physiologically active form. The proportion of bound cortisol is highly variable and dependent on concentrations of cortisol and binding proteins. This study is aimed to compare methods for free cortisol measurement for identification of adrenal insufficiency in septic shock.

Methods:
Serum specimens were collected from septic shock patients in the ICU. A protein-free serum was obtained by ultrafiltration using Centrifree Ultrafiltration Devices (Merck Millipore Ltd). Serum free cortisol was then estimated by measuring cortisol in ultrafiltrate. The methods for cortisol measurement include immunoassay (IA) with and without extraction of serum ultrafiltrate, liquid chromatography tandem mass spectrometry (LC-MS/MS), and these results were compared with salivary free cortisol by LC-MS/MS.

Results:
This is a pilot clinical study with 10 septic shock patients. Results of serum free cortisol by IA and LC-MS/MS as percentage of serum total cortisol is seen in Table 1.
Table 1. Mean % serum free cortisol by IA and LC-MS/MS
Serum Free Cortisol by IA 28.0 ± 18.2
Serum Free Cortisol by IA after Extraction 22.1 ± 12.5
Serum Free Cortisol by LC-MS/MS 13.8 ± 8.7
Salivary Free Cortisol by LC-MS/MS 13.2 ± 12.4
(n = 10, mean ± SD)

Serum free cortisol by IA without extraction of ultrafiltrate (y) correlates very well with IA following extraction of ultrafiltrate (x) ($y = 0.6838x + 17.572$, $R2 = 0.9835$) and is comparable with LC-MS/MS method (x) ($y = 0.4242x + 13.667$, $R2 = 0.9142$). The serum cortisol IA on ADVIA Centaur is precise, CV < 7%. Further study will be done to determine precision of ultrafiltration step and define reference intervals of serum free cortisol by IA without extraction of ultrafiltrate.

Conclusions:
Direct measurement of serum cortisol by IA using ultrafiltration is a simple and reliable method to estimate free cortisol for adrenal function assessment in septic shock.

ALISA ABOZINA

Supervisor: Dr. Randy Gascoyne
Session: Basic Sciences

"Identifying a novel gene fusion partner in burkitt lymphoma"

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Background/objectives:
The incidence of lymphoma is 20 per 100,000 people per year in the Western world, with 95% of these being of B-cell origin. WHO lymphoma classification distinguishes 15 types of B-cell lymphoma, with differences in pathogenesis, treatment, and prognosis. Reciprocal chromosomal translocations involving a proto-oncogene and one of the Immunoglobulin (IG) loci are often seen, and are a hallmark of many types of B-cell lymphoma. Burkitt lymphoma is an aggressive lymphoma with cytogenetic abnormalities often including a t(8;14)(q24;q32) translocation, which results in the fusion of the MYC proto-oncogene on chromosome 8 with the constitutively-active IG heavy chain promoter on chromosome 14. In this Burkitt lymphoma case, a t(14;15) representing a novel IGH fusion partner was present in addition to the t(8;14). Identifying this novel fusion partner may provide insight into the pathogenesis of disease, and represents a potential novel therapeutic target.

Methods:
Fluorescence in situ hybridization (FISH) using break-apart probes was used to identify the novel fusion partner on chromosome 15.

Results and Conclusions:
FISH break-apart probes localized the novel fusion partner on chromosome 15 to a region of approximately 220 kilobases near the TCF12 gene. The product of the TCF12 gene is a 682 amino acid transcription factor, and is a member of the class I helix-loop-helix proteins, which have direct E-box DNA binding ability. Overexpression of TCF12 has been associated with metastasis of colorectal cancer. TCF3, a homolog of TCF12, and its negative regulator ID3, were found to be mutated in 70% of sporadic Burkitt lymphoma cases; TCF3 activates a pro-survival pathway in Burkitt lymphoma. Further research is needed to elucidate the role of TCF12 in Burkitt lymphoma pathogenesis.

NEGAR FARZAM-KIA

Supervisor: Dr. Jacqueline Quandt
Session: Basic Sciences

"Antioxidant influence on the expression of antigen-presenting molecules at the blood brain barrier"

AUTHORS:
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Background/objectives:
Multiple sclerosis (MS) is a chronic inflammatory, neurodegenerative disease. MS is associated with significant infiltrations of autoreactive T cells through the blood brain barrier (BBB), which target and attack the myelin covering of neurons in the central nervous system (CNS). This causes problems in efficient signal transduction along neurons, and can result in a variety of symptoms. Symptoms range from extreme fatigue and blurred vision, to complete vision loss and motor impairments. Experimental autoimmune encephalomyelitis (EAE) is the murine model of MS and has been studied to elucidate the pathogenesis of the disease. Tempol is a stable free radical that acts as a scavenger, neutralizing the effects of less stable free radicals, such as reactive oxygen species or nitric oxide. Interestingly, Tempol administered to mice either before or after EAE induction has a protective effect. That is, EAE scores reflecting symptom severity are decreased, especially in prophylactic Tempol administration. However, the exact mechanisms of action remains unknown. It has been shown that endothelial cells in vitro can be induced by Interferon-y (IFNy) to express HLA-DR, an MHC-II molecule, which plays an important role in antigen presentation to promote inflammation. Preliminary data has shown that IFNy does indeed induce HLA-DR expression in endothelial cells in vitro, which is then decreased with Tempol. Given this information, we hypothesize that Tempol may exert its neuroprotective effect by decreasing HLA-DR expression in endothelial cells of the BBB.

Methods:
Human cerebral microvascular endothelial cells (hCMEC/D3) were seeded and cultured until about 95-100% confluent, receiving a media change every two days. When confluent, cells were treated with 200 u/mL IFNy, with or without Tempol at 100, 200, and 500 uM. Other treatments included IFN+ Betaseron or Copaxone, established drugs used in MS therapies. Unstimulated cells served as controls. At 48 hours post-treatment, cells were trypsinized, processed, and stained for HLA-DR for flow cytometry, or harvested, quantified for the amount of protein, and prepared for Western blot analysis.

Results:
Preliminary results show that Tempol limits the induced expression of HLA-DR in hCMEC/D3 cells. Flow cytometry has shown this to be dose dependent, such that Tempol 500 uM decreases HLA-DR expression more than at 200 uM and 100 uM.

Conclusions:
Preliminary results indicate the Tempol may in fact play a neuroprotective role by reducing MHC-II expression, and thus limiting leukocyte infiltration past the BBB and into the CNS. This effect is similar to, but not as strong as, that of Betaseron on HLA-DR expression. Future experiments may examine the effects of Tempol on BBB tight junction proteins, endothelial cell adhesion molecules, and BBB permeability *in vitro*.

SONIA KUNG

Supervisor: Dr. Wan L. Lam
Session: Basic Sciences

"Aberrant expression levels of the protein kinase MARK2 functionally contribute to lung cancer"

AUTHORS:
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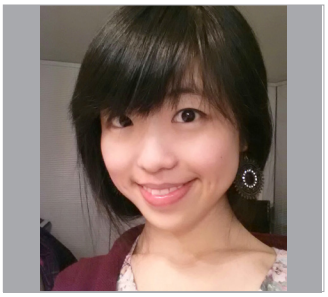
Background/objectives:
In Canada, lung cancer is the first cause of all cancer morbidities. An integrated understanding of the molecular signature of the disease drives the discovery of therapeutic targets that ameliorate this poor prognosis. With this objective, we recently identified that Microtubule Affinity Regulating Kinase 2 (MARK2) mRNA is overexpressed in 30% of lung adenocarcinoma in relation to genomic and epigenomic alterations that encompass copy number gain and hypermethylation. MARK2 regulates target substrates functioning in cell cycle and neurogenic microtubule dynamics associated with Alzheimer's disease. However, the role of MARK2 in lung cancer is unknown. This study investigates the consequences of changes in MARK2 expression in lung cancer.

Methods:
Briefly, MARK2 protein levels were experimentally modulated in lung adenocarcinoma tumour cells and immortalized normal lung cells with lentiviral knockdown and inducible overexpression systems. Cell growth in MARK2-modulated and control cells were measured using proliferation and anchorage-independence assays. Cancer pathways regulated by MARK2 were identified by measuring transcription factor activities through luciferase reporter assays. Key proteins from the identified pathways were further validated by western blot.

Results:
We determined that lung cancer cells exhibited significantly less cell growth upon experimental reduction of the protein (p-value<0.05). Furthermore, normal lung cells achieved greater proliferation with ectopic expression of MARK2. We report for the first time that MARK2 regulates the cancer pathway NF-kappaB. MARK2 overexpression downregulated NF-kappaB, and the opposite relationship was concordantly observed in MARK2 knockdowns. Western blot determined that MARK2 regulates non-canonical NF-kappaB activities related to DNA damage repair via phosphorylated p65.

Conclusions:
Our results substantiate that digressions in MARK2 protein levels impact cell growth. MARK2 likely promotes lung cancer pathogenesis by atypical regulation of cellular pathways, including DNA damage repair pathways linked to NF-kappaB signaling. Elucidating the role of MARK2 in DNA damage repair may provide novel insights to combat chemotherapy resistance.



MENG YING (MELODY) LU

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Investigating analysis parameters for deep sequencing-based mitochondrial DNA somatic point mutation rate assay"

AUTHORS:
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Background/objectives:
Mitochondria, contain many copies of their own DNA. Due to its proximity to the electron transport chain, a primary producer of free radicals, mitochondrial DNA (mtDNA) is particularly susceptible to oxidative damage. This may result in the build-up of mtDNA mutations and can lead to mitochondrial dysfunction, which contributes to aging. Our lab has developed a deep sequencing based assay to quantify age related mtDNA mutation rates, however both the PCR and sequencing steps introduce artificial mutations at a rate higher than the rate of mtDNA mutations due to aging in blood. To overcome this limitation, our assay uses degenerate primers to distinguish PCR or sequencing background errors from true biological mtDNA mutations. Degenerate primers label every mtDNA molecules prior to DNA amplification and deep sequencing such that each mtDNA strand is labeled with a unique Primer ID. This allows the grouping of the sequencing reads according to the original DNA molecule they came from into groups. Mutations that were present in an original DNA molecule should be present in all the amplified strands, while mutations seen in only some of the reads are likely PCR polymerase and/or sequencing errors. The same PCR/Sequencing induced mutations can occur within a group, at the same position multiple times, such that criteria must be established to distinguish this from the "true" mutations. For example, not using groups with lower numbers of templates, and not counting mutations present in a group at low frequency are two methods of filtering PCR/sequencing induced mutations from "true" mutations. The goal of this project is to determine the minimum number of sequencing reads needed in each group, and the minimum frequency at which a mutation should to be present in a group, in order to be yield robust and reproducible results.

Methods:
Deep sequencing data were collected and analyzed from a plasmid sample, a muscle sample and two blood samples. The plasmid sample is the negative control; it is clonal DNA that theoretically contains no mutations. The muscle sample is from an individual with a mutation in the polymerase gamma gene, causing it to introduce mtDNA mutations; this is our positive control,.Each sample was sequenced several times within the same run, and then again in a separate run. Different cutoffs for minimum sequencing reads per group and mutation frequency were tested, and both intra- and inter-run variabilities were calculated to evaluate the cutoffs.

Results:
No pattern was observed for intra- and inter-run variability at low group number and low mutation frequency cutoffs. However ≥5 sequencing reads per group and ≥75% mutation frequency, changes in intra- and inter-run variability were reduced.

Conclusions:
These preliminary data suggested that these cutoffs are optimal for the analysis of blood samples using this assay. However, more data are needed to validate the cutoffs.

ADAM YU

Supervisor: Dr. Jacqueline Quandt
Session: Basic Sciences

"Characterizing the expression and localization of the neuroprotective protein arnt2 in cellular models using immunocytochemistry "

AUTHORS:
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Background/objectives:
Multiple sclerosis (MS) is a neurological disease of the central nervous system that can result in extreme fatigue, vision and speech impairment, cognitive dysfunction and paralysis due to demyelination and neurodegeneration. Previously, our lab has shown that the administration of an antioxidant drug in animal models of MS showed a decrease in the incidence and progression of the disease altering the expression of neuronal PAS domain protein 4 (Npas4) and aryl hydrocarbon receptor nuclear translocator (ARNT2). The role of these proteins in neuronal and axonal development, survival and involvement in cognition and learning has been described.This suggests that ARNT2 and Npas4 may have a functional role in neurodegenerative processes in MS and may be potential therapeutic targets. Our objective is to characterize some of the factors that may regulate ARNT2 expression in neurons, specifically, to study the effects of oxidative stress and its correlation with viability.

Methods:
Primary cortical neurons were dissected and cultured in neuronal basal media for 14 days in vitro. After 14 days the cells were stimulated with hydrogen peroxide for various time points. Arnt2 expression was determined through western blotting while Arnt2 expression and viability was determined through immunocytochemistry and through a lactate dehydrogenase assay.

Results:
Arnt2 expression determined by a western blot found that expression decreased with time after exposure to hydrogen peroxide from 0 to 16 hours. Data generated from the LDH assay determined that cell viability decreased with time after 2 hours suggesting that there is a possible correlation between low Arnt2 expression and cell death. Through immunocytochemistry, it was determined that high Arnt2 expressing cells decreased with exposure time to hydrogen peroxide as low Arnt2 expressing cells increased. High Arnt2 and low Arnt2 expressing cells were foudn to be significantly different between unstimulated neurons and neurons that have been treated with hydrogen peroxide at 4 hours. As well, a peak in Arnt2 at 0.5 hours correlates with a peak in Arnt2 found in our western blot at the same time point. At this same time point it was also found that there was lag period in exposure to oxidative stress where the cells remained viable. This suggests that an increase in Arnt2 expression may lead to an increase in cell viability when exposed to oxidative stress.

Conclusions:
Cell death increases over time as ARNT2 expression decreases when exposed to oxidative stress (hydrogen peroxide) suggesting there is a correlation between cell viability and ARNT2 Expression.

ADAM ZIADA

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Relationships between aging, HIV status, body mass index, and blood mitochondrial DNA somatic point mutations"

AUTHORS:
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Background/objectives:
Despite the success of antiretroviral therapy at preventing AIDS, people living with HIV seem to experience accelerated aging (cardiovascular disease, non-AIDS related cancers, and neurodegenerative diseases). Mitochondria, the powerhouse of the cell, contain their own mitochondrial DNA (mtDNA) which encodes proteins that are crucial to energy production. The oxidative stress theory of aging states that the accumulation of mtDNA mutations over time leads to tissue aging. Somatic mtDNA point mutations are implicated in many age-associated diseases such as those seen in HIV. The virus itself, as well as antiretroviral drugs, can increase oxidative stress, and may also contribute to accelerated aging.
Objective: In this pilot study, we investigated whether blood somatic mtDNA point mutations show a relationship with biological aging, HIV infection status or body mass index (BMI).

Methods:
Somatic mtDNA point mutations are challenging to quantify because of their low frequency. We developed a novel next generation mtDNA sequencing assay that can distinguish true mtDNA mutations from background ones. The strategy is based on primers containing degenerate bases (assigned at random), generating 67 million unique mtDNA "tags" that are integrated into the DNA sequence of each molecule of mtDNA before they are amplified for sequencing on the 454 platform. This allows us to distinguish the original mtDNA template molecules from one another. Sequences are grouped based on their "tags", creating consensus sequences of what the "original mtDNA molecules". True mutations should be present in ~100% of sequences with the same "tag", since they are theoretically derived from the same molecule of mtDNA, while background mutations (Taq errors or sequencing miscalls) should not.
Study design: Blood samples collected from HIV+ and HIV- individuals aged 1-75 years and enrolled in the CARMA cohort study were studied, along with clonal plasmid DNA (no mutations control) (n=5). Somatic mtDNA point mutations were characterized and compared in the following four groups: A: HIV+, aged 6-14 years (n=10); B: HIV+, aged 52-68 years (n=10); C: HIV-, aged 6-14 years (n=10); and D: HIV-, aged 52-68 years (n=10).
Statistics: The association between mtDNA somatic substitution mutations and BMI was analyzed by spearman correlation while the association with age (old vs. young) or HIV status (HIV+ vs. HIV-) was investigated through two-group comparisons (Mann-Withney test).

Results:
A significant association between increased mtDNA somatic substitution mutations and increased BMI in adults (R= 0.35, p=0.007). There was a tendency toward older subjects (HIV+ and HIV- together) having more mtDNA mutations (p=0.051). However, there was no relationship detected between HIV status and mtDNA mutations (p=0.41).

Conclusions:
Older age and excess weight have been associated with increased oxidative stress. Our preliminary data would be consistent with this being reflected by mtDNA mutations. Although HIV infection increases inflammation and oxidative stress, we did not detect a difference in our small sample. However, many potential confounding factors were not considered here. A larger sample size needs to be tested to confirm these findings while adequately controlling for important covariables.

ALYM ABDULLA

Supervisor: Dr. David Pi
Session: Clinical Sciences

"Linking testing-to-clinical goals - A risk stratified process re-design scheme towards improving manual blood film review (MBFR) services in a large tertiary hospital laboratory"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
A hematopathologist's review and comment (HPCOM) on blood film can be of critical importance in the initial diagnostic work up of a hematological disease. Recent feedback from the clinical users verified that HPCOM information is generally not available for timely clinical decision making and patient management. This paper presents the development and implementation of a process redesign project, aiming to improve the turn-around-time (TAT) of critical HPCOM results to meet clinical user's expectation in a high volume large tertiary hospital laboratory.

Methods:
The key components of the logic model include: (1) A risk stratification scheme to separate blood film morphology abnormalities - (i) minor/low diagnostic specificity entities, as Disease Associated Abnormalities (DAA), or (ii) major/clinical significant entities, as Disease Defining Abnormalities (DDA). For DAA, the use of an intelligent data manager (MiddlewareTM), permits the development of customized alert codes/decision functions for some numerical abnormalities, as a diagnostic aid, based on pre-defined logics in the transactions of laboratory information to clinical users. (2) For DDA, the implementation goal is to upkeep/advance the diagnostic acuity of the front-line technologists. An Inter-professional Learning (IPL) model is designed to foster a codependent learning opportunity between the technologists and pathologists. The combined strategies aim to cope with the technologists' workload in managing DAA cases, while enhancing their core competence in recognizing critical morphological abnormalities and refer them to a pathologist for review on a priority basis. Anonymous LIS data at Vancouver General Hospital before and after program implementation were extracted and analyzed for performance evaluation.

Results:
Despite an increase in CBC test volume of 6.5% (23,843 vs. 25,380 respectively) in the study period (January - 2013 and 2014), before and after program implementation, the rate of MBFR by technologists remained constant (4,337 vs. 4,666; 18.2% vs. 18.4% respectively). The HPCOM cases declined substantially (1,024 vs. 839; 4.3% vs. 3.3% of total respectively). A significant reduction in TAT for HPCOM was evident: (1) Median TAT (14.6 vs. 6.8 hours respectively; $p<0.0001$), (2) TAT cut-off ≤ 6 hours (27.3% vs. 46.1% respectively; $p<0.0001$.

Conclusions:
The standard MBFR process relies on a sequential transactional pathway, resulting in significant time delay in the final release of HPCOM information to clinical users. This is a proof-of-concept model which demonstrated that risk stratification/prioritization and knowledge sharing in a case-based learning environment are effective strategies and can improve the clinical goals of MBFR services.

CYRUS CHEHROUDI

Supervisor: Dr. Bruce Verchere
Session: Basic Sciences

"Blockade of IL-1 signalling improves islet amyloid-induced glucose intolerance and prevents early amyloid formation"

AUTHORS:
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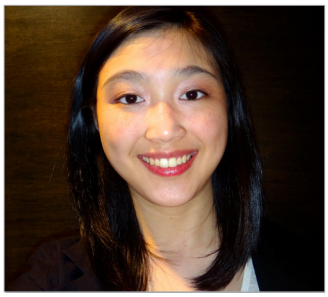
Background/objectives:
Islet amyloid polypeptide (IAPP) is co-secreted with insulin by beta cells. Aggregation of IAPP to form amyloid contributes to beta cell dysfunction in patients with type 2 diabetes, a condition also characterized by elevated islet expression of pro-inflammatory cytokines such as interleukin-1 beta (IL-1 beta) that impair the processing and secretion of insulin. Because proIAPP processing occurs in parallel with that of proinsulin and accumulation of partially processed proIAPP may contribute to its aggregation, we sought to determine the effects of IL-1 on glucose homeostasis, islet amyloid formation, and proIAPP processing.

Methods:
Wild-type or transgenic mice with beta cell expression of human IAPP (hIAPPTg/o, either lean or obese due to Avy expression at the agouti locus) were injected daily with IL-1 receptor antagonist (IL-1Ra, 50 mg/kg) or PBS for 8 weeks starting at 16 weeks of age. Glucose tolerance was assessed by intraperitoneal glucose tolerance test and islet amyloid prevalence was determined using thioflavin-S. MIN6 beta cells were incubated with recombinant IL-1 beta and IAPP was detected by western blot. mRNA expression in MIN6 cells was measured by qRT-PCR.

Results:
IL-1Ra significantly improved glucose tolerance in both lean and obese hIAPPTg/o mice (lean: AUC=710±180 vs. 1150±450; obese: AUC=1360±110 vs. 880±70; $p<0.05$) and reduced amyloid prevalence in lean (35% vs. 2%; $p<0.05$) but not obese mice, suggesting that IL-1 signalling may be important in modulating early amyloid formation. Treatment of MIN6 cells with 100 pg/ml IL-1 beta for 24 h induced expression of the chemokines Ccl2 and Cxcl1 but had no effect on expression of Ins2, IAPP, or the genes encoding the prohormone processing enzymes PC1/3 or PC2. IL-1 beta impaired proIAPP processing as determined by a 50% reduction in the ratio of mature to total IAPP. IL-1 beta also caused a dose-dependent decrease in the proportion of active PC2 protein relative to total PC2, suggesting a possible mechanism for impaired processing of proIAPP.

Conclusions:
Our data suggest that IL-1 mediates IAPP-induced islet dysfunction and promotes early amyloid formation by a mechanism that may involve impaired IAPP processing. Anti-IL-1 therapies may improve islet function in type 2 diabetes in part by reducing amyloid formation.



DAPHNE CHEUNG

Supervisor: Dr. Blake Gilks
Session: Clinical Sciences

"Expression profiles of p53 protein in the five most common subtypes of epithelial ovarian carcinoma"

AUTHORS:
Daphne W.Y. Cheung

AFFILIATIONS:
Department of Pathology and Laboratory Medicine

Background/objectives:
Mutations in the TP53 gene are the most common molecular alteration in epithelial ovarian cancer. A missense mutation in the TP53 gene confers increased stability of the p53 protein and has been correlated with strong and diffuse immunolabeling. Conversely, a nonsense mutation in the TP53 gene results in a truncated p53 protein and has been correlated with a complete absence of immunostaining. In this study, we investigate the immunohistochemical expression profiles of p53 protein among the 5 most common subtypes of epithelial ovarian cancer - clear cell, endometrioid, high-grade serous carcinoma (HGSC), low-grade serous carcinoma (LGSC), and mucinous - using a large sample of cases from 28 different institutions across the province.

Methods:
FFPE ovarian tumor samples from 770 patients were available in the pathology archives of 28 different institutions. Tissue microarrays were constructed and analyzed by immunohistochemical staining for p53 expression. Expression patterns were evaluated using a three-tier scoring system to represent a complete absence of expression, normal expression, and overexpression of the p53 protein.

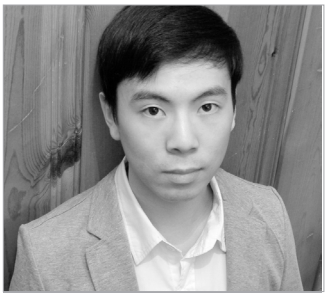
Results:
HGSCs show aberrant p53 expression (i.e. either a complete absence of expression or overexpression) in 89% of the cases ($P < 0.001$), while non-HGSC cases show aberrant p53 expression in only 25%-44% of the cases.

Conclusions:
HGSCs are significantly associated with aberrant p53 expression. Further directions for this study can assess the clinical outcomes and risk factors for the patients with a complete absence of expression to see how these cases compare with the patients showing an overexpression or normal expression of p53.

BRIAN CHO

Supervisor: Dr. Decheng Yang
Session: Basic Sciences

"Functional analysis of death-associated protein 5 (DAP5) N- and C-terminal cleavage products during coxsackievirus B3 infection"



AUTHORS:
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Background/objectives:
Coxsackievirus B3 (CVB3) is a major cause of viral myocarditis. The CVB3 genome is a positive, single-stranded RNA that is translated using an internal ribosome entry site (IRES)-mediated mechanism as opposed to cap-dependent translation. Upon infection, CVB3 induces cellular stress that shuts down global cap-dependent translation and promotes IRES-mediated translation. It was previously determined that death-associated protein 5 (DAP5), an IRES-translation initiation factor and homolog of eukaryotic translation initiation factor 4G (eIF4G), is cleaved by viral protease 2A at amino acid Glycine 434 into 45 kDa N-terminal and 52 kDa C-terminal truncated forms. The function and cellular distribution of these two cleavage fragments remains unknown. Our experimental aim is two-fold: to determine the cellular localization of N- and C-terminal truncated forms after cleavage; and to determine the effect of cleavage products on the translation of IRES-containing target genes.

Methods:
IN VIVO: To confirm DAP5 cleavage in vivo, mice were infected with either sham or CVB3. Mouse heart tissue was harvested at 3 and 9 days post infection. Tissue was lysed and analyzed on western blot by probing for DAP5. NUCLEAR EXTRACTION: HeLa cells were transfected with Flag-tagged full-length DAP5, Flag-tagged N-terminal, or HA-tagged C-terminal gene constructs for 48 hours. Nuclear and cytoplasmic proteins were isolated from cell lysates. The proteins were then analyzed by western blot using anti-Flag and anti-HA antibodies. CONFOCAL: Confocal microscopy was used to visualize the cellular localization of DAP5 and its cleavage products after immunochemistry staining. Flag-tag and HA-tag were probed using ALEXA 594 and ALEXA 488.

Results:
IN VIVO: DAP5 is shown to be cleaved in vivo at 9 days post CVB3 infection in mice heart tissue. NUCLEAR EXTRACTION: Nuclear extraction results demonstrate that full-length DAP5 mainly resides in the cytoplasm; N-terminal DAP5 is present in similar levels in both the nucleus and cytoplasm; C-terminal DAP5 is largely localized to the cytoplasm. CONFOCAL: Confocal imaging confirmed similar findings as that of western blot analysis.

Conclusions:
The cleavage products of DAP5 show differing localization compared to full-length DAP5. Particularly, N-terminal DAP5 shows translocation to the nucleus. To determine the effect of the cleavage products on target gene translation, bicistronic luciferase reporter assays will be performed using plasmids containing the IRES of DAP5 translational target genes after co-transfection with a plasmid overexpressing either the N- or C-terminal fragments. Finally, the experiments will be conducted in human cardiomyocytes.

HELEN FU

Supervisor: Dr. Andrew P. Weng
Session: Basic Sciences

"The role of RUNX transcription factors in T-cell acute lymphoblastic leukemia (T-ALL) leukemogenesis"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
RUNX proteins (RUNX1-3) encode context-dependent transcriptional activators/repressors which bind DNA as part of an obligate heterodimer with CBF-beta. Recently described mutations in RUNX1 occur in roughly 15-18% of T-cell acute lymphoblastic leukemia (T-ALL) patients, a subset of which are predicted to generate dominant-negative proteins that interfere with normal RUNX transcriptional activity, thus implying RUNX1 may act as a tumour suppressor in T-ALL. We discovered in a genetic screen, however, that T-ALL in mice is frequently associated with gain-of-function RUNX mutations, suggesting that RUNX may instead be an oncogene. Moreover, we have generated preliminary data showing that lentiviral shRNA-mediated knockdown of RUNX1 and/or RUNX3 compromises growth/survival in several human T-ALL cell lines, consistent with the idea that RUNX proteins supports oncogenic phenotypes in this context. We next sought to determine if enforced expression of RUNT, a truncated RUNX1 polypeptide consisting of only the N-terminal DNA binding domain and which resembles polypeptides predicted to occur by mutation, would produce similar phenotypes. We hypothesize that RUNT will function in a dominant-negative fashion and phenocopy the effect of RUNX1 knock-down.

Methods:
Two lentivirus vectors were made, one an empty vector and one containing the C-terminally truncated RUNX1 (RUNT domain only). Both contain GFP for tracking growth within a mixed population of transduced and untransduced cells. We utilized HPBALL, a well-characterized human T-ALL cell line, for these studies. On day 5 post transduction, we also sorted transduced cells to obtained purified populations for protein analysis. We performed immunoblots for proteins known to be regulated by RUNX1 including c-MYC, p21WAF1/CIP1, and p27KIP1. We also assessed expression of IGF1R and IL7R by flow cytometry on day 8 post-transduction as these have also been reported to be regulated by RUNX1.

Results:
Western blots showed that c-MYC and p27KIP1 protein levels both decreased in RUNT-expressing cells. There was a slight decrease in expression levels of IL7R for the RUNT-transduced cells, yet no difference was seen for IGF1R. We observed no significant growth disadvantage associated with enforced RUNT expression as compared to empty vector controls.

Conclusions:
The RUNT construct exhibited only a subset of the phenotypes associated with RUNX1 knockdown, suggesting that the biology of spontaneous truncating RUNX1 mutations is distinct from that of complete loss of RUNX1 function (as modeled by shRNA-mediated knockdown).

SANDY LOU

Supervisor: Dr. Stephen Yip
Session: Basic Sciences

"Novel isocitrate dehydrogenase-1 (IDH1) mutation in adult medulloblastoma"

AUTHORS:
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AFFILIATIONS:
[1]University of British Columbia,
2Division of Neuropathology,
Department of Pathology

Background/objectives:
Medulloblastoma is a highly invasive embryonal neuroepithelial tumor that arises in the cerebellum. It is predominantly a brain tumor of childhood (accounts for 20% of all pediatric CNS tumors) with a slight increase in incidence between 20 to 24 years of age but rare after the fourth decade of life. The much lower incidence in adults may suggest a different tumorigenesis or even entity from the pediatric population. This may have implications in treatment and prognosis as management for the adult population is often extrapolated from pediatric studies. Isocitrate dehydrogenase (IDH) is an enzyme that catalyzes the oxidative decarboxylation of isocitrate to 2-oxoglutarate in the glycolytic pathway and plays an important role in cellular protection against oxidative stress. IDH1 mutations have been previously characterized in other CNS tumors such as glioblastoma and oligodendrogliomas but not medulloblastoma. It has been speculated that IDH1 mutations render cells more susceptible to oxidative DNA damage, thus, associated with positive prognosis. This study aims to further characterize the genetic properties of adult medulloblastoma, firstly by looking at IDH1 and IDH2.

Methods:
DNA was extracted from fresh frozen tissue or FFPE tissue of adult medulloblastoma specimens using commercially-available kits. DNA extracted were then quantified and subsequently amplified via PCR. PCR product was confirmed via agarose gel electrophoresis. Bidirectional Sanger sequencing of IDH1 and IDH2 was performed using different primers. DNA sequences obtained were then analyzed using Mutation Surveyor.

Results:
Analysis revealed that 10 out of the 21 samples of adult medulloblastoma contain the same single base mutation in codon 132 from CGT to CAT leading to a missense mutation from arginine to histidine.

Conclusions:
IDH1/2 mutations have not been observed in pediatric medulloblastoma. The novel discovery of IDH1 mutation suggests that adult medulloblastoma may indeed be a separate entity from the pediatric population which can profoundly affect treatment and prognosis. IDH1 mutations may also serve as a prognostic indicator as in other CNS tumors.

PAULINA PIESIK

Supervisor: Dr. Honglin Luo

"Regulation of mutant p53-R175H by autophagy"

AUTHORS:
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AFFILIATIONS:
[1]Centre for Heart Lung Innovation, Department of Pathology and Laboratory Medicine

Background/objectives:
Tumour suppressor protein p53 is a critical regulatory molecule involved in cell cycle regulation and apoptosis. It acts as a tumour suppressor by inhibiting cell division when cellular stresses such as DNA damage are present. p53 promotes DNA repair, cell cycle arrest, or cell death depending on the degree of damage. p53 is also known to regulate autophagy, a "self-eating" behaviour that targets aged or nonfunctional organelles and protein aggregates for lysosomal degradation. Further, mutations in p53 are frequently associated with tumourigenicity. The point mutation variant p53-R175H is a dominant-negative mutant associated with increased metastasis and pro-oncogenic gene expression. This mutant p53 is known to form insoluble protein aggregates resulting from significant conformational changes in its three dimensional structure. Our hypothesis is that stably expressed mutant p53-R175H forms insoluble co-aggregates that are targets for degradation by the autophagy pathway.

Methods:
UMSCC-1 cells, derived from a squamous cell carcinoma cell line lacking endogenous p53 expression, were stably transfected with wild-type p53 or p53-R175H for this study. Autophagy was induced by 24 hr starvation in Hank's Balanced Salt Solution, treatment with 100nM rapamycin, or over-expression of the autophagic adaptor p62. Cells were treated with the lysosome inhibitor chloroquine (100μM) for 24 hrs to inhibit autophagy. Protein levels of p53 were assessed using Western Blot analysis. Activation or inhibition of autophagy was monitored by evaluating protein levels of the autophagy substrates p62, Nbr1, and LC3-I/-II by Western Blotting.

Results:
We found that treatment with starvation or 100nM rapamycin for 24 hrs reduced protein levels of mutant but not wild-type p53. We then over-expressed p62 to see if p62-mediated selective autophagy is responsible for this decrease. Over-expression of p62 did not change the protein levels of either wild-type or mutant p53, suggesting that p62 is not involved in regulating p53-R175H. Interestingly, lysosome inhibition by chloroquine increased the levels of both wild-type and mutant p53, suggesting a possible role for lysosome activity in regulating protein levels of wild-type p53 and p53-R175H.

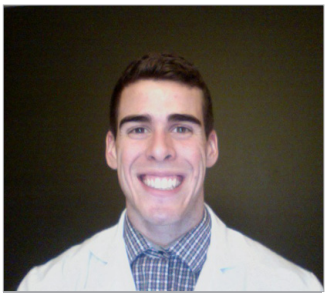
Conclusions:
Our preliminary findings demonstrate that p53-R175H is a potential target for autophagic degradation. Future therapies can be designed to selectively target p53-R175H for degradation, restore the function of wild-type p53, and decrease the oncogenicity of tumors carrying p53-R175H.

This research is supported by the Canada-China Joint Grant from the Canadian Institutes of Health Research. P. Piesik is supported by the Department of Pathology and Laboratory Medicine Summer Student Fellowship Program.

MITCHELL WEBB

Supervisor: Dr. Patrick Tang
Session: Basic Sciences

"Identification of viral markers for monitoring water quality"



AUTHORS:
Mitchell Webb[1], Miguel Uyaguari-Diaz[1,2], Matthew Croxen[1,2], Natalie Prystajecky[1,2], Judy Isaac-Renton[1,2] and Patrick Tang[1,2]

AFFILIATIONS:
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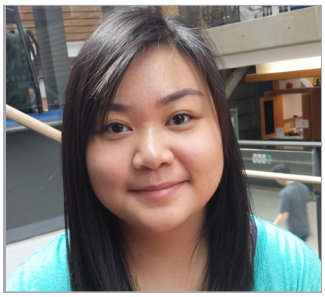
Background/objectives:
Water quality is both a public health and an economic concern. Unfortunately, our current model of water quality monitoring, the E. coli count, is inherently slow and, with regards to the source of contamination, relatively uninformative. By replacing this culture-based test with a molecular assay targeting genomic markers of viruses, bacteria, and protists, it is possible to develop a more rapid and accurate test. This project focused on determining specific viral markers that could be used towards this end.

Methods:
Water samples were taken from three sites: rural, agricultural, and protected. Within each site there was a nested control. Samples were filtered and viral particles were extracted using tangential flow filtration with a 100kDa filter followed by ultracentrifugation to concentrate the retentate. These samples were prepared for sequencing using Illumina's Nextera XT protocol. Output data from the MiSeq next generation sequencer was quality filtered, matched to a viral database using the USEARCH algorithm, and compared via principal component analysis and heatmaping. Filtering statistics, match results, and diversity scores were calculated and recorded for each sample.

Results:
In the pre-filtered data, there was a large discrepancy in the total number of reads per sample; agricultural samples had 25000 reads while urban samples had as few as 96. Additionally, the percent of reads that successfully passed quality filtering ranged from 86 - 6% between samples. In general, samples from RNA viruses that required pre-amplification before sequencing had reads with a 22% lower pass rate than corresponding DNA samples where pre-amplification wasn't required. Using USEARCH to match filtered reads against a viral database resulted in varying success, with a mean match rate of 16.5% and a standard deviation of 17.7%. The average Shannon Index per sample, after normalization by random sampling, was 4.91 with SD of 0.306.

Conclusions:
Further investigation, data collection and analysis is required in order to identify useful viral markers for an assay to investigate water quality. With improved sampling methods and viral extraction techniques, better quality reads can be attained from sequencing. This will improve the the specificity of match results to the viral database. Additionally, as more viruses are sequenced the database will become more comprehensive. Considering that only a small fraction of the viral community has been sequenced to date, further work could investigate the usefulness of unmatched Oportational Taxonomic Units in their ability to predict contamination.



DENISE WONG

Supervisor: Dr. Colby Zaph
Session: Basic Sciences

"Temporal and spatial analysis of mesenchymal progenitor cells in intestinal inflammation and regeneration"

AUTHORS:
Denise Wong, Menno Oudhoff, Colby Zaph

AFFILIATIONS:
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Tissue restoration following intestinal inflammation involves interactions between intestinal epithelial cells (IECs) and mesenchymal stromal cells (MSCs). Recent studies have identified a role for a specific population of MSCs, termed mesenchymal progenitor cells (MPCs), in tissue regeneration of skeletal muscle and skin. A population of MPCs positive for platelet-derived growth factor alpha (PDGFR-α) was also identified in intestinal issues. We used genetically modified mice that express green fluorescent protein (GFP) when cells express PDGFRα to investigate the localization and activity of PDGFRα+ MPCs during intestinal inflammation caused by dextran sodium sulphate (DSS)-induced colitis and whole-body irradiation. In naive mice, we found that PDGFRα + MPCs are distributed along the top of intestinal crypts as well as in the lamina propria in between crypts. During DSS-induced colitis, PDGFRα+ MPCs accumulate and proliferate in the early injury phase and decrease in number and proliferative activity during the regeneration phase. Interestingly, no accumulation and proliferation of PDGFRα+ MPCs were noted in mice treated with whole-body irradiation. These findings provide the first analysis of the temporal and spatial dynamics of PDGFRα+ MPCs in intestinal inflammation. Future studies will address whether these cells play a functional role in inflammation and regeneration and may identify a novel cellular target for new treatments for inflammatory bowel diseases

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