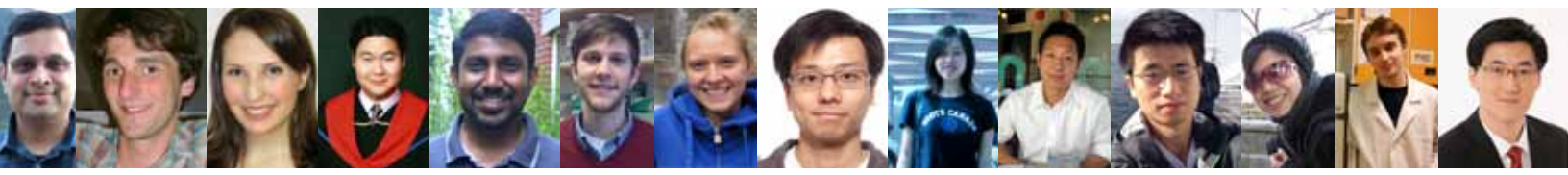
PATHOLOGY DAY 2013

abstract book | posters and oral presentations



a place of mind

THE UNIVERSITY OF BRITISH COLUMBIA

pathology laboratory medicine

ACKNOWLEDGEMENT

PATHOLOGY**DAY**
www.pathology.ubc.ca

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2013 edition. Mike Allard and Adeline Chan were both instrumental in the organization of this event. 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.

Finally, a sincere thanks to staff who kindly assisted with technical and administrative support throughout the day.

We hope you enjoy Pathology Day 2013.

Jacqueline Quandt, Michael Nimmo and Avi Ostry

Co-Chairs, Pathology Day 2013

ORGANIZING COMMITTEE

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

Michael Nimmo, BA, BSc Honours, LLB, MD (UBC), & LMCC, FRCPC, Clinical Associate Professor

Avi Ostry, Clinical Associate Professor, UBC

SPECIAL THANKS TO THE MANY FACULTY WHO HAVE CONTRIBUTED AS JUDGES OR CHAIRS FOR PATHOLOGY DAY:

Mike Allard
Sachiv Sheth
William Schreiber
Deborah McFadden
Jay Kizhakkedathu
David Schaeffer
Cornelia Laule
Valerie White
Doug Filipenko
Vicky Monsalve
Hélène Côté
Avi Ostry
Mike Nimmo
Diana Ionescu
Decheng Yang
Cedric Carter
Colby Zaph
Maria Issa
David Granville
Torsten Nielsen
Haydn Pritchard

WE WOULD ALSO LIKE TO RECOGNIZE THE TECHNICAL AND ADMINISTRATIVE SUPPORT OF SEVERAL DEPARTMENT MEMBERS INCLUDING:

Debbie Bertanjoli
Andrew Leung
Jennifer Xenakis
Jenny Tai
Julianna Li
Helen Dyck

A MESSAGE FROM THE HEAD

WELCOME

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 scholarly activities, including basic investigative, translational and clinical-applied research, performed by our trainees and, by extension, our faculty. This year, we are pleased to include the 2012 Pathology Summer Studentship Recipients and highlight their research in the poster session. This gathering also provides a perfect venue to recognize and acknowledge the many outstanding contributions by members of the department over the past year.

Pathology Day also serves another very important function. Specifically, it is an opportunity to get together to socialize and learn 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. A new feature of the program this year, where select members of our department present their own work, will help assist in gaining 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. David Hardwick (Department of Pathology and Laboratory Medicine, University of British Columbia) will give the James Hogg Lecture, while Dr. Douglas Smith (Department of Neurosurgery, University of Pennsylvania) is our Keynote Speaker. I extend my thanks and gratitude to Drs. Jacquie Quandt, Avi Ostry and Mike Nimmo, Co-Chairs of Pathology day, as well as all the other individuals for their efforts in organizing this year's event.

Hope you enjoy the day,

Michael F. Allard, Head

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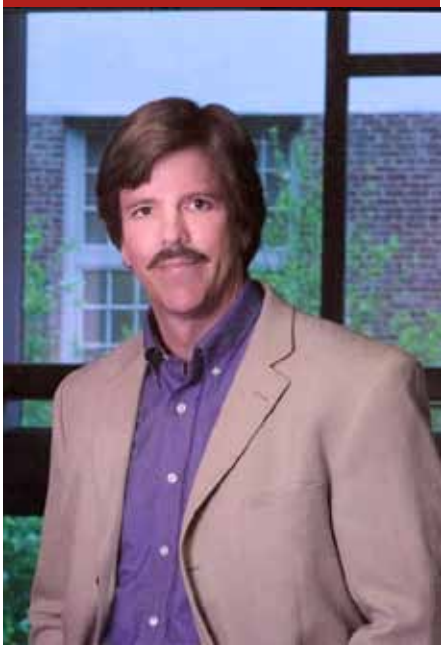
Keynote Speaker



DOUGLAS H. SMITH

Robert A. Groff Professor of Neurosurgery, Dept. of Neurosurgery, Perelman School of Medicine, University of Pennsylvania

"Tackling Concussion: Neuromechanics and Neuropathology"

Douglas H. Smith serves as Director of the Center for Brain Injury and Repair (CBIR) and is the Robert A. Groff Endowed Professor and Vice Chairman for Research and Education in Neurosurgery at the University of Pennsylvania. Penn's multidisciplined CBIR includes over twenty-five principal investigators and their laboratory staff collectively studying mechanisms, diagnosis and potential treatments of traumatic brain injury. Dr. Smith is also director of a multi-center U.S. National Institutes of Health (NIH) program grant on mild traumatic brain injury and oversees an NIH brain injury training grant. Over the last 20 years, Dr. Smith has devoted his full-time efforts to neurotrauma research following completion of fellowships in both molecular biology and neurotrauma at the University of Connecticut. His laboratory investigates the effects of mechanical stretch of axons that results in either damage or growth. They have found that rapid stretch during brain trauma selectively injures axons in the white matter. In turn, aberrant accumulation of proteins in the damaged axons can lead to pathologic changes similar to those found in Alzheimer's disease. In addition, Dr. Smith's laboratory has also recently discovered that slow continuous stretching of axon tracts in culture can stimulate enormous growth, creating transplantable living nervous tissue constructs. These tissue engineered constructs have shown promise for repairing large lesions in the nervous system. These collective efforts have resulted in over 150 published reports.

GUEST SPEAKERS

DR. RANDY GASCOYNE
Clinical Professor, Pathology - UBC; Hematologist, BCCA

"Impacting the lives of lymphoma patients through translational research"

DR. HÉLÈNE CÔTÉ
Associate Professor, Pathology - UBC; Member, Women's Health Research Institute and Centre for Blood Research

"HIV and antiretroviral therapy: how does it affect cellular aging?"

DR. ED PRYZDIAL
Clinical Professor, Pathology - UBC; Scientist, Canadian Blood Services

"Dissolving clots: a clarifying mechanism"

James Hogg Lecture

DAVID F. HARDWICK

Professor Emeritus, Dept. of Pathology and Laboratory Medicine, University of British Columbia

"Investing in the Future of Pathology"

Professor Emeritus David F. Hardwick has been involved with UBC for the best part of six decades, and during this time been party and witness to much change. He remains an energetic presence on campus and influential leader in the organization of Pathology and affairs of the university, especially those concerning the Faculty of Medicine.

Dr. Hardwick has been a major influence behind the faculty's decision-making. Over his long career, he was Head of Pathology, the Associate Dean of Research and Planning and continues to the present as Special Advisor on Planning.

Dr. Hardwick has a strong interest in inter-institutional systems, and can claim a lot of credit for optimizing the relationship between UBC and its distributed teaching hospitals.

Dr. Hardwick has seen his profession adapt through many social changes, including the current growth in information technology. Early on he grasped that potential and examines how cutting-edge technologies can best be employed for learning, research and enhancing community and practice. He also conceived and helped develop a free online resource for fellow practitioners called The Knowledge Hub for Pathology. It provides up-to-date knowledge to help ensure best possible practices, currently serves more than 20,000 practitioners in 168 countries and continues rapid growth.

Dr. Hardwick is a leader of his profession and strong proponent of high professional standards. He is currently Secretary of the International Academy of Pathology.

Dr. Hardwick has received many accolades from respected professional organizations, and is a popular guest speaker with numerous publications to his name.

CONFERENCE OUTLINE

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

7:30am	**Breakfast**
8:00am	**JAMES HOGG LECTURE:** Dr. David Hardwick - "Investing in the future of pathology" (Lecture Theatre)
9:00am	**GUEST SPEAKER:** Dr. Randy Gascoyne - "Impacting the lives of lymphoma patients through translational research"

Resident Oral Session (Multipurpose Room)

Abstract	9:30am-10:30am
#1.	Lien Hoang: Histotype-genotype correlation in 36 high-grade endometrial carcinomas.
#2.	Martin Hyrcza: Emergency frozen sections at Vancouver General Hospital - a review and creation of a teaching module for residents.
#3.	Joyce Leo: Histological subtyping of ampullary carcinoma - does it matter
#4.	Ananta Gurung: Centralized breast cancer bio-marker testing: a value-added role in guiding patient management
#5.	Nouf Hijazi: Dysplastic features in congenital nevi.

Graduate Student Oral Session (Lecture Theatre)

Abstract	9:30am-10:30am
#17.	Kevin Yang: The battle is on: small molecule EPI-002 versus aberrant androgen receptor activity in castration-resistant prostate cancer.
#18.	Bryant Harbourne: Hypoxia induced secreted proteins and the pre-metastatic niche.
#19.	Ada Kim: The role of heme oxygenase-1 (HMOX1) in breast cancer metastasis.
#20.	Momir Bosiljcic: Myeloid-derived suppressor cell accumulation in secondary target organs is tumour hypoxia driven and promotes a higher metastatic potential in breast cancer.

10:30am - 11:00 am	**Break**
11:00am - 11:20am	**GUEST SPEAKER:** Dr. Hélène Côté - "HIV and antiretroviral therapy: how does it affect cellular aging?"

Resident Oral Session (Multipurpose Room)

Abstract	11:20am - 12:35pm
#6.	Tareq Mohammad: Breast carcinoma with choriocarcinomatous features: a case report and review of literatures.
#7.	Jason Morin: Significance of dissecting stromal mucin in intraductal papillary mucinous neoplasms.
#8.	Peyman Tavasolli: Non-destructive microfluidic cell-based assay for determining cell deformability as a function of epithelial-to-mesenchymal transition.
#9.	Patrick Wong: Retrospective review of IgG4-related disease with serum IgG subclass patterns.
#10.	Ramesh Saeedi: Relationship between cholesterol efflux and high-density lipoproteins in patients with familial hypercholesterolemia.
#11.	Sophia Wong: Mineralocorticoid-derived indices in adrenal vein sampling for primary aldosteronism subclassification.

Graduate Student Oral Session (Lecture Theatre)

Abstract	11:20am - 12:35pm
#21.	David Twa: Tumour microenvironment-altering genomic rearrangements in B-cell lymphomas.
#22.	Jesse Olson: Programming of glucose homeostasis by gestational exposure to folic acid and vitamin B12 imbalance.
#23.	Gabriel Fung: Coxsackievirus B3 disrupts cytosolic stress granules by viral protease 3C.
#24.	Ada Leung: Silencing of PAPSS1 (3'-phosphoadenosine 5'-phosphosulfate synthase 1) potentiates cisplatin activity against non-small cell lung cancer.

12:30pm - 2:00pm	**Lunch and poster session (UBC Medical Student & Alumni Centre, 2750 Heather Street)**
2:15pm - 2:35pm	**GUEST SPEAKER:** Dr. Ed Pryzdial - "Dissolving clots: a clarifying mechanism"

Resident Oral Session (Multipurpose Room)

Abstract	2:45pm - 3:45pm
#12.	Michael Payne: Evaluation of two chromogenic media for the isolation and identification of urinary tract pathogens (UTP).
#13.	Amanda Wilmer: Shigella flexneri serotype 1 infections in men who have sex with men in Vancouver, Canada.
#14.	Veronica Hirsch-Reinshagen: Clinicopathological correlations in frontotemporal dementia and amyotrophic lateral sclerosis due to C9orf72 repeat expansion.
#15.	Peter Schutz: Suprasellar hemangioblastoma during pregnancy – a case analysis.
#16.	Maxim Signaevski: IDH1 R132H immunohistochemical staining in laboratories across Canada showed accurate and reproducible results.

Graduate Student Oral Session (Lecture Theatre)

Abstract	2:45pm - 3:45pm
#25.	Melissa McConechy: Intratumoural heterogeneity and the detection of plasma circulating tumour DNA in endometrial carcinomas.
#26.	Erica Osbourne: Small molecule EPI-002 inhibits transcriptional activities of androgen receptor and truncated variants in castrate-resistant prostate cancer.
#27.	Alon Hendel: Granzyme B releases vascular endothelial growth factor from extracellular matrix stores and induces vascular permeability in vivo.
#28.	Amal El-Naggar: YB-1 contributes to sarcoma metastasis via translational activation of HIF1α.

3:45 - 4:00pm	**Break**
4:00pm - 5:00pm	**KEYNOTE SPEAKER:** Dr. Douglas Smith - "Tackling concussion: neuromechanics and neuropathology" (Lecture Theatre)
6:00pm	**Awards Gala Dinner at Shaughnessy Restaurant**

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ABSTRACT #

LIEN HOANG

Supervisor: Dr. Cheng-han Lee
Session: Clinical Sciences

"Histotype-genotype correlation in 36 high-grade endometrial carcinomas"

Background/objectives:
Serous carcinomas and endometrioid/clear cell carcinomas of the endometrium are genetically distinct tumor types with differing prognoses. The distinction between these tumor types can be difficult, particularly in high-grade cases.

Methods:
36 high-grade endometrial carcinomas (J Path 2012;228:20-30) were included; 23 endometrioid/clear cell genotype (PTEN and ARID1A mutations or either one without TP53 and PPP2R1A mutations) and 13 serous genotype (TP53 and/or PPP2R1A mutations without ARID1A or PTEN mutations). 8 pathologists reviewed representative online slides and rendered diagnoses before and after receiving p53, p16 and ER immunoprofiles. Kappa statistics for histotype-genotype concordance were calculated for each pathologist.

Results:
The average kappa values for histotype-genotype concordance was 0.56 (range: 0.31-0.67) based on morphology alone, and improved to 0.68 (range: 0.54-0.81) after immunoprofile consideration (p=0.009). Genotype incompatible diagnoses were rendered by at least 2 pathologists in 12 of 36 cases (33%) (3 with 2/8, 2 with 3/8, 2 with 4/8, 3 with 6/8, 1 with 7/8 and 1 with 8/8). Diagnostic disagreement occurred in 6 endometrioid and 6 serous genotype cases. The problematic scenarios identified are discussed below.

Conclusions:
While the majority of morphologic diagnoses are genotype-concordant, genotype-incompatible diagnoses are made in a significant subset of cases. p53 immunohistochemistry improved histotype-genoype concordance.

Based on our findings, we have derived the following recommendations:

I) In tumors with predominantly or exclusively papillary/villoglandular growth pattern: **p53 IHC is recommended** in cases with more than low-grade (intermediate to high-grade) nuclear features and/or increased mitotic activity. A mutated p53 IHC (diffusely all or none) supports a serous carcinoma. p16 was not as helpful and dissuaded some pathologists away from a diagnosis of serous even though p53 IHC was abnormal.

II) In tumors with predominantly solid/confluent growth pattern: **p53 IHC result in the solid area is not informative**. There is a need to identify and examine for better differentiated areas (which may need additional sections) and interpret p53 IHC in these areas.

AUTHORS:
Lien N Hoang[1], Melissa K McConechy[1]Martin Koebel[2], Guangming Han[3], Marjan Rouzbahman[3], Ben Davidson[4], Julie Irving[1], Rola Ali[1], Sam Leung[1], Esther Oliva[5], Marisa R Nucci[6], Rob A Soslow[7], David G Huntsman[1], C Blake Gilks[1] and Cheng-Han Lee[1]

AFFILIATIONS:
[1]University of British Columbia; [2]University of Calgary; [3]University of Toronto; [4]Norwegian Radium Hospital, Norway; [5]Massachusetts General Hospital, Boston; [6]Brigham and Women's Hospital, Boston; [7]Memorial Sloan-Kettering Cancer Center, New York

MARTIN HYRCZA

Supervisor: Dr. C. Blake Gilks
Session: Clinical Sciences

"Emergency frozen sections at Vancouver General Hospital - a review and creation of a teaching module for residents"

AUTHORS:
Martin Hyrcza[1], Nicholas Sunderland[2], C. Blake Gilks[1]

AFFILIATIONS:
[1]Anatomical Pathology, Department of Pathology and Laboratory Medicine, University of British Columbia; [2]General Pathology, Department of Pathology and Laboratory Medicine, University of British Columbia

Background/objectives:
Unexpected, emergency frozen section is one the anatomic pathologist's most demanding and stressful tasks, yet residency training programs do not prepare residents in this area. Emergency frozen sections are infrequent and tend to involve a distinct set of conditions, different from the usual types of specimens received for frozen section analysis. Review of literature reveals a dearth of published data on this topic. Specifically, the frequency and types of specimens submitted for emergent frozen sections are have not been reported in recent publications.

Methods:
We retrospectively analyzed the emergency frozen sections performed on weekends for non-neuropathology cases at Vancouver General Hospital, a large metropolitan teaching hospital, between June 2010 and Aug 2012. Eighteen cases were identified for which the on-call pathologist was requested to perform emergent frozen section analysis.

Results:
The indications for the emergent frozen sections were: diagnosis (lesion identification) in 14/18 cases (78%), margin assessment in 3/18 cases (17%), lymph node assessment (6%), and transplant tissue quality assessment in 3/18 cases (17%) (one case involved both margin and diagnosis). Of the eighteen cases, six (33%) were performed for emergent general surgery cases such as perforated or obstructed viscus, severe bleeding from a mass, and one case of VIPoma causing severe hypokalemia. Four cases (22%) involved assessment of tissue quality assessment prior to transplantation. Four cases (22%) involved mediastinal lesions causing either superior vena cava syndrome or acute cardiac symptoms. Three cases (17%) involved orbital or base of skull masses. The final case involved acute spinal cord compression (6%).

Conclusions:
The review of emergent frozen sections revealed a distinct set of situations for which emergent frozen section analysis is called for. These include: 1) acute presentations of gastrointestinal mass lesions due to obstruction, perforation, bleeding or other symptoms requiring same day surgery; 2) mediastinal masses causing SVC syndrome or acute cardiac symptoms; 3) orbital or skull base masses presenting with acute vision loss or other neurological deficits; 4) spinal cord compression syndrome; and 5) assessment of transplant organ quality. Identifying these classes was then used to develop an online educational module to specifically prepare pathology residents for this challenging task.

JOYCE LEO

Supervisor: Dr. David Schaeffer
Session: Clinical Sciences

"Histological subtyping of ampullary carcinoma - does it matter?"

AUTHORS:
Joyce M Leo[1], Steve Kalloger[1], Renata Peixoto[2], Doug L Webber[1], David A Owen[1], Dan Renouf[2], David F Schaeffer[1]

AFFILIATIONS:
[1]Division of Anatomical Pathology, Vancouver General Hospital, Department of Pathology & Laboratory Medicine, The University of British Columbia; [2]BC Cancer Agency, Vancouver, Canada

Background/objectives:
Ampullary carcinomata (AC) are defined as tumors that completely replace, surround or are centered on the ampulla of Vater by the World Health Organization (WHO). Anatomically, the ampulla of Vater is the dilated conduit resulting from the union of the common bile duct (CBD) and the major pancreatic duct. As such, AC are separated histologically based on epithelial origin, intestinal (AC-I) or pancreatobiliary (AC-PB) subtypes. Immunohistochemically, AC-I have shown cytokeratin 20 (CK20) and caudal type homeobox 2 (CDX2) positivity, while AC-PB are cytokeratin 7 (CK7) positive. Morphologic, immunohistochemical and molecular differentiation of AC-I and AC-PB have been well documented, however, prognostic significance of histologic subtype has yet to be established. The purpose of this proposed study is to identify whether there is prognostic significance between the different histological subtypes of AC and to assess if patients with either subtype benefited from pancreas or intestinal specific chemotherapeutic treatment.

Methods:
Patients who underwent either an ampullectomy or pancreatoduodenectomy for ampullary carcinoma at Vancouver General Hospital between 1997 and 2011 were identified retrospectively. Demographic information, as well as pathological variables were collected. Outcome/survival information, was recorded. The tumours were classified as intestinal (CK7-/CK20+/Cdx2+) and pancreaticobiliary (CK7+/CK20-/Cdx2 -) type. All lesions were further evaluated in regards to their mismatch repair status by staining representative sections for the hMLH-1, hMSH2, hMSH6 and hPMS2 gene products.

Results:
Initial analysis of the study cohort included 33 [mean age 65.5 ± 10.4 years (range 41 to 82 years); 15:18; f:m] with sixteen patients (n=16) demonstrating an AC-I subtype and seventeen patients (n=17) classified as AC-PB. CK7 staining was positive in AC-PB (100%) and AC-I (53.3%, p-value 0.0019). CK20 staining showed greater positivity in AC-I (93.75%) than AC-PB (29.41%, p-value 0.0002). CDX2 staining was positive only in AC-I (93.75%, p-value <0.0001). MLH-1 deletion was detected in 2 cases of AC-PB. Tumor size for AC-PB (mean size 2.36 cm, CI 1.9;2.81,) was generally larger compared to AC-I (mean size 1.73 cm, CI 1.25;2.22, p value >0.06). Both histological subtypes were proportional for lymphovascular invasion (p-value 0.69). Analysis for additional tumor characteristics, patient specific chemotherapeutic treatment and overall survival are ongoing.

Conclusions:
Ampullary carcinomata, defined by the refined criteria, are a heterogenous group. Initial analysis indicates a lack of utility of CK7 in differentiating between AC-I and AC-PB and the potential of CDX2 being more useful, suggesting the need for a multi-marker panel. AC-PB tumors generally present larger suggesting detection at a later stage and therefore potentially conferring a poorer prognosis. Although this study is still ongoing and a complete data set for outcome analysis is not available, subtyping AC appears important in delineating prognostic significance and possible therapeutic interventions.

Resident | Oral Session

ANANTA GURUNG

Supervisor: Dr. Amir Rahemtulla
Session: Clinical Sciences

"Centralized breast cancer bio-marker testing: a value-added role in guiding patient management"

AUTHORS:
Ananta Gurung, Gregory Naus, Diana Ionescu, Malcolm Hayes, Kathy Ceballos, Tadaaki Hiruki, Helga Klein-Parker, Thomas Thomson, Dirk van Niekerk, Chen Zhou, Amir Rahemtulla

AFFILIATIONS:
BC Cancer Agency, Department of Pathology

Background/objectives:
A characteristic of breast bio-marker testing in British Columbia is its extensive centralization at the British Columbia Cancer Agency (BCCA). For each block submitted to BCCA for bio-marker testing a hematoxylin and eosin (H&E) stained slide is reviewed, and if deemed appropriate, assays for estrogen receptor, progesterone receptor and Human Epidermal Growth Factor Receptor 2 (HER2) are performed. Since bio-marker testing centralization a number of discrepant results were encountered, so this study was conducted to determine reasons for discrepancies.

Methods:
Prospectively (over one year period), bio-marker testing was performed on 2439 patients. 253 cases involved solely testing for HER2 gene expression with fluorescence in situ hybridization, so the total number of cases examined was 2186. Discrepant diagnoses and reasons for disagreements were recorded.

Results:
The discrepancy rate from the 2186 cases was approximately 0.9% (19 cases): 11 cases were initially reported as invasive breast carcinoma, 7 cases as ductal carcinoma in-situ (DCIS) and 1 case as benign. 12/19 (63%) of discrepant diagnoses arose from core biopsies. 8 invasive cases were changed to a non-invasive lesion (DCIS, atypical apocrine adenosis, sclerosing papillary lesion, sclerosing adenosis and complex sclerosing lesion) and 3 invasive cases were changed to another malignancy (pleomorphic lobular carcinoma, diffuse large B-cell lymphoma and plasma cell myeloma). Of 7 discrepant cases involving DCIS, 3 showed evidence of invasion, whereas the remaining 4 cases did not show evidence of DCIS (instead showed flat epithelial atypia, atypical ductal hyperplasia, atypical papillary lesion and benign breast parenchyma). 1 case which was initially reported as benign showed invasive carcinoma.

Conclusions:
Centralization of bio-marker assays offers many benefits, including accredited standardized staining protocols and consistency with interpretation. It is an invaluable practice that may drastically affect patient care, ensuring patients are managed appropriately.

NOUF HIJAZI

Supervisor: Dr. Richard Crawford
Session: Clinical Sciences

"Dysplastic features in congenital nevi"

ABSTRACT #5

Background/objectives:
To highlight the extent of dysplastic features present in congenital nevi.

Methods:
A total of 55 nevi with congenital features were evaluated. Inclusion criteria for congenital features were:mandatory single-filing of melanocytes infiltrating the interstitium of the reticular dermis and perivascular cuffing or infiltration of preexisting dermal structures such as nerve and arrector pili muscle. Exclusion criteria were: blue nevi, nevi from the head and neck and incisional biopsies. Criteria used to define dysplastic features were those described in the WHO 1989 consensus.

Results:
18% (11/55) of nevi with a congenital pattern fulfilled criteria for dysplastic nevi. Of the 30 congenital nevi with a junctional component, 11 (37%) fulfilled criteria for dysplastic nevi.

Conclusions:
A significant minority of congenital nevi will fulfill the histological criteria for dysplastic nevi. Therefore, caution must be taken to avoid misdiagnosing this subcategory as dysplastic nevi, consequently alarming the clinicians and patients and leading to unnecessary further surgery and follow up.

AUTHORS:
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AFFILIATIONS:
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TAREQ MOHAMMAD

Supervisor: Dr. Diana Ionescu
Session: Clinical Sciences

"Breast carcinoma with choriocarcinomatous features: A case report and review of literature"

AUTHORS:
Tareq Mohammad[1], Rola Ali[1], Malcom M. Hayes[2], Diana N Ionescu[2]

AFFILIATIONS:
Departments of Pathology, [1]; Vancouver General Hospital and University of British Columbia, Vancouver, BC, Canada, [2]; BC Cancer Agency and University of British Columbia, Vancouver, BC, Canada

Background/objectives:
Primary breast carcinoma with choriocarcinomatous features (BCCF) is a very rare breast malignancy that has to be differentiated from metastatic choriocarcinoma, which is also extremely rare. This distinction is important, as the treatment of these entities is entirely different. Several other entities are included in the differential diagnosis.

Methods:
The patient's file was reviewed along with histological and immunohistochemical slides. Literature search through PubMed to obtain all reported (BCCF) cases was performed. These cases where reviewed.

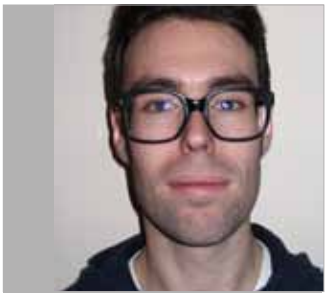
Case:
We report a rare case of primary breast carcinoma with choriocarcinomatous features (BCCF) in a premenopausal woman, who presented at an early stage and had a good outcome after a long term follow up. The histological diagnosis was confirmed on the basis of history, histology (excisional biopsy) and immunohistochemistry, negative metastatic work up as well as a long term follow up with serial tumor markers and imaging studies for a period of 8 years. Morphologically the tumor showed nests and sheets of large markedly pleomorphic, mitotically active cells with numerous tumor giant cells in a hemorrhagic and necrotic background. Immunohistochemistry showed the tumor cells to be positive for B-hCG, hPL, Keratin and CEA and negative for ER and GCDFP-15.

Conclusions:
Primary breast carcinoma with choriocarcinomatous features (BCCF) is a distinct variant of metaplastic breast cancer. It is an extremely rare entity that should be distinguished from metastatic choriocarcinoma because of the significant differences in treatment regimen between the two. This is achieved by thorough clinical investigations to exclude a primary focus of choriocarcinoma of the genital tract and immunohistochemistry to rule out other differential diagnoses. Long-term follow up is also important in reaching this goal.

JASON MORIN

Supervisor: Dr. David Schaeffer
Session: Clinical Sciences

"Significance of dissecting stromal mucin in intraductal papillary mucinous neoplasms"



AUTHORS:
Jason Morin[1] and David Schaeffer[1]

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

Background/objectives:
Intraductal papillary mucinous neoplasm (IPMN) is a mucin-producing, grossly visible neoplasm arising in the pancreatic ductal system. Subtypes are classified clinically according to duct involvement: main duct (MD), branch duct (BD), and mixed. MD-IPMNs may progress to invasive carcinoma (either tubular adenocarcinoma or colloid carcinoma). IPMNs produce large amounts of mucin into the lumen of the involved duct which can result in elevation of intraluminal pressure. This may lead to disruption of the duct and extrusion of mucin into the stroma. Acellular stromal mucin pools are pitfalls in evaluation of IPMNs and can be misdiagnosed as colloid carcinoma. Colloid carcinomas can be distinguished; however, by the presence of neoplastic epithelium floating within stromal mucin. The distinction between the two is important - the overall five year survival for colloid carcinomas while relatively good at 60% (vs 12% for tubular adenocarcinomas) is significantly lower than that of IPMNs, which in some studies approach 90 - 100%. This study attempts to better define the histological and demographic charateristics of IPMNs with extravasation of mucin in order to identify other features helpful in delineating them from an invasive carcinoma.

Methods:
Ten cases of IPMN with extravasated mucin were located via search of local pathology databases. These cases were age/sex matched to cases of IPMNs without stromal mucin, as well as invasive carcinomas, giving a total of three groups. Demographic information as well as histologic attributes (tumour size, IPMN sub-type, grade, presence of background pancreatic intraepithelial lesions (PanIN)) were collected and compared between the groups.

Results:
There was no male or female preponderance for disease within the groups, however there was trend towards increased age going from IPMNs to IPMNs with stromal mucin and invasive carcinoma (65.6 vs 68.1 vs 69.1 years). IPMNs with stromal mucin tended to be larger than those without extravasated mucin (3.04 cm vs 2.31 p=0.35) and were significantly smaller than invasive carcinoma (3.04 cm vs 5.25 p=0.009). The presence of high grade dysplasia increased across the three groups (IPMN to IPMN with stromal mucin to carcinoma) - 12% vs 20% vs 90% (p=0.60 and 0.0004 respectively). The presence of background PanIN was similar between all three groups (80 vs 76 vs 82%), however the invasive carcinoma group had a significantly increased rate of high grade PanIN (47% vs 10% in IPMNs with stromal mucin vs 5% in IPMNs).

Conclusions:
IPMNs with stromal mucin have histologic characteristics that suggest an intermediate phenotype lying somewhere between IPMNs without extravasated mucin and invasive carcinomas. Further work is needed to identify underlying molecular mechanisms to account for these differences. The clinical/prognostic significance of the observed differences between IPMNs and IPMNs with stromal mucin is unknown, however long term follow-up and patient outcome data may help to delineate whether dissecting stromal mucin without free-floating neoplastic cells is a high risk feature of IPMNs.

PEYMAN TAVASSOLI

Supervisor: Dr. Peter Black
Session: Clinical Sciences

"Non-destructive microfluidic cell-based assay for determining cell deformability as a function of epithelial-to-mesenchymal transition"

AUTHORS:
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Background/objectives:
The routine identification of circulating tumor cells (CTC) is based on cell surface expression of epithelial markers in the cancer cells. It is likely, however, that a subset of the most invasive CTC has lost expression of these epithelial markers in the process of epithelial-to-mesenchymal transition (EMT), a critical process that enables cancer cells to detach from their primary site of growth, to invade and to metastasize. We have therefore developed a microfluidic device for the identification of CTC based on cell deformability. Here we aim to demonstrate that cell deformability depends on EMT status in bladder cancer cells.

Methods:
We have established a microfluidic technique for measuring the deformability of single cells. Cells are infused into a microfluidic channel and through a narrowing that requires cell deformation for passage. Using precisely controlled pressure, the cortical tension is determined using the liquid-drop model. Zeb-1, a mediator of mesenchymal differentiation, was silenced (shRNA) in the highly invasive and mesenchymal cell line UC-13, while E-cadherin, the prototypical maker of epithelial differentiation, was silenced in UC-1. Differences in cortical tension and in invasive ability (matrigel invasion assay) were compared between these cells and the scramble controls.

Results:
UC-13 cells were less invasive after silencing of zeb1, and the mean cortical tension increased from 490 pN/μm to 837 pN/μm, representing a 1.7-fold increase in stiffness. Similarly, UC-1 cells were more invasive after silencing of E-cadherin, and the mean cortical tension decreased from 1198 pN/μm to 774 pN/μm, representing a 1.5-fold decrease in stiffness.

Conclusions:
The non-destructive microfluidic cell-based assay enables us to measure the cortical tension of a variety of cells. We were able to demonstrate that cortical tension was inversely related to invasiveness of a bladder cancer cells, and both were dependent on EMT status. We therefore believe that EMT status will influence passage of CTC through our microfluidic cell sorting device, and aim to demonstrate this in ongoing work. This could be also potentially be used as a complementary test for clinical cytology, especially in difficult samples such as urine cytology.

PATRICK WONG

Supervisor: Dr. Andre Mattman, Dr. Nadine Urquhart
Session: Clinical Sciences

"Retrospective review of IgG4-related disease with serum IgG subclass patterns"

AUTHORS:
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Background/objectives:
Immuoglobulin G4-related disease (IgG4RD) is a recently described clinicopathologic entity which typically affects middle-aged to elderly men, consists of tumefactive lesions of one or more sites, and generally without constitutional symptoms. Autoimmune pancreatitis is the prototypic syndrome; however, involvement of nearly every organ system has been reported, including the hepatobiliary tract, salivary gland, orbit, lymph node, retroperitoneum, aorta, soft tissue, central nervous system, breast, kidney, prostate, gastrointestinal tract, lung, and skin. Typical laboratory findings include raised serum immunoglobulins (IgG-total, IgG4, IgE), positive autoantibodies (rheumatoid factor, anti-nuclear antibody), normal or mildly elevated lactate dehydrogenase, and peripheral eosinophilia. However, serum IgG subclass patterns and parameters have not been firmly established for the prediction of IgG4RD. Despite the diverse clinical manifestations, histopathological findings in all tissues are remarkably similar and remain as the gold-standard for IgG4RD diagnosis with the IgG4 immunohistochemical stain as one of the diagnostic criteria. IgG4RD can mimic certain malignancies and autoimmune diseases, and thus, poses a diagnostic challenge. In this proposed study, we hope to collect relevant clinical data and observe IgG subclass level patterns in relation to IgG4RD.

Methods:
The health records of all patients with elevated serum IgG4 levels were reviewed to obtain information about exposure to immunomodulatory medications, related markers of IgG4RD, and whether the diagnosis of IgG4RD was made. Inclusion criteria: initial IgG4 serum level >1.25 g/L, measured between January 1, 2010 and December 31, 2012. Exclusion criteria: not meeting inclusion criteria, patients on any immunomodulatory agents that are known to affect serum IgG levels at the time of IgG subclass testing, or insufficient clinical data for analysis. Cases were then stratified into IgG4RD positive and negative groups. T-tests and ROC curves were used for statistical analysis.

Results:
In general, the absolute IgG4 concentration and the IgG4/IgG-total ratio were significantly higher in the disease positive than negative cases. Additionally, the absolute IgG-total and IgG2 concentrations were significantly greater in the disease positive than negative cases. This is especially true for the hepatobiliary-pancreas phenotypes. However, the IgG1/IgG-total ratio was found to be significantly lower in the disease positive than negative cases. This is especially true for the head and neck and hematolymphoid phenotypes. IgG3 had no difference between the disease groups. ROC curves of IgG1/2/3/4/total absolute concentrations showed an AUC of 54/72/67/88/69% respectively. ROC curves of IgG1/2/3/4 to IgG-total ratios showed an AUC of 22/47/56/89% respectively. ROC curve of IgG4/IgG1 ratio showed an AUC of 88%. An absolute IgG4 concentration >2.5g/L, IgG4/IgG-total ratio >16%, and IgG4/IgG1 ratio of >0.4 each gave a sensitivity of 92% for the diagnosis of IgG4RD with respective specificities of 74/82/82%.

Conclusions:
In this retrospective study, we confirmed that raised absolute IgG4 concentration and the IgG4/IgG-total ratio had the greatest ROC AUC as serum markers for IgG4RD. Slight improvements in the AUC, and in prediction of organ involvement, were noticed with incorporation of the IgG1/IgG-total ratio and the absolute IgG-total and IgG2 concentrations, although these improvements require prospective validation.

RAMESH SAEEDI

Supervisor: Dr. Jiri Frohlich
Session: Basic Sciences

"Relationship between cholesterol efflux and high-density lipoproteins in patients with familial hypercholesterolemia"

AUTHORS:
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Background/objectives:
Heterozygous familial hypercholesterolemia (hFH) is an autosomal dominant disorder characterized by an elevated plasma concentration of low-density lipoprotein cholesterol (LDL-C) at birth, and increased risk of atherosclerosis and premature cardiovascular disease (CVD). There is a wide variation in the onset and severity of atherosclerotic disease in these patients, indicating that other genetic and/or environmental factors may play a role. The cholesterol efflux capacity (CEC), a measure of how well patient's plasma removes cholesterol from lipid-loaded cells, is one such factor and is considered a key process that protects against development of atherosclerosis. The aim of this study is to investigate whether hFH patients with history of CVD will have significantly lower CEC than FH patients without history of CVD.

Methods:
THP-1 cell line, derived from human acute monocytic leukemia cells, were differentiated into macrophages by treatment with phorbol myristate acetate , labeled with [3H]-cholesterol and incubated for 18 hours with liver X receptor agonist TO-901317 to up-regulate the expression of ABCA1 transporters. Cholesterol efflux was measured after incubating apoB-depleted plasmas with activated labeled human THP-1 for a further 2 hours. Media and cells were collected for counting of. CEC was defined as a proportion of radioactivity in medium divided by total radioactivity in medium plus cells. Data were normalized to simultaneously measured efflux in pooled plasma from healthy controls and high-density lipoprotein cholesterol (HDL-C) concentration of individual patients.

Results:
Data from 11 healthy controls, 17 hFH subjects without CVD, and 7 hFH subjects with CVD were compared. hFH subjects with CVD tended to be older and had a higher proportion of males than hFH subjects without CVD although the differences did not reach significance. After normalizing for variation across assays and individual differences in HDL concentration, there were no significant differences in CEC among healthy controls, hFH subjects without CVD, and hFH subjects with CVD.

Conclusions:
The preliminary data suggest that CEC does not appear to be a significant factor in the development of CVD in people with hFH.

SOPHIA WONG

Supervisor: Dr. Daniel T. Holmes
Session: Clinical Sciences

"Mineralocorticoid-derived indices in adrenal vein sampling for primary aldosteronism subclassification"

AUTHORS:
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Background/objectives:
Primary aldosteronism (PA), the most common curable form of hypertension, consists of two main subtypes: aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia (BAH). Subtype determination requires the evaluation of two parameters in blood specimens collected from adrenal vein sampling (AVS). First is the selectivity index (SI), which serves as a marker of adrenal vein cannulation success; second is the lateralization index (LI), which distinguishes APA from BAH. Although cortisol is traditionally used for SI and LI calculations, a transient elevation of its level occurs in most patients who undergo AVS, with impact on the interpretation of both indices. We therefore investigate whether other adrenal steroids, namely the mineralocorticoid precursors, may serve as an adjunct to, or may even replace, cortisol in SI and LI determinations.

Methods:
22 subjects (16 APA and 6 BAH) were included in the study. Their AVS specimens were analyzed for cortisol (F), aldosterone (A), deoxycorticosterone (DOC), 18-hydroxy-deoxycorticosterone (18OHDOC), corticosterone (B), and 18-hydroxycorticosterone (18OHB) concentrations via the multiplex steroid liquid chromatography-tandem mass spectrometry (LC-TMS) assay developed in-house at St. Paul's Hospital. SIs and LIs were calculated using DOC, 18OHDOC, B, and 18OHB in lieu of F.

Results:
DOC-, B-, and 18OHDOC-derived SIs were found to be better determinants of adrenal vein catheterization than F-derived SI in the pre-adrenocorticotropic hormone (ACTH) phase of the procedure, and were as good of an index as F in the post-ACTH phase. The absolute change in SI with ACTH administration was also significantly higher in these mineralocorticoid-based SIs than that calculated from F ($p<0.001$ for all).

Regarding lateralization, F-derived LI remained the best determinant of PA subtype under basal conditions, followed by B-, DOC-, and 18OHDOC-derived indices, although there were no statistical differences. Post-ACTH stimulation, B-calculated LI identified 1 additional case as compared to the other 3 indices. Concurrent analysis of pre- and post-ACTH data revealed that both F- and DOC-based LIs correctly determined PA subtype in 100% of cases, while the diagnostic rate of 18OHDOC- and B-based LIs was 96%.

Conclusions:
DOC-, B-, and 18OHDOC-derived SIs are better and more sensitive markers than the F-calculated SI for the ascertainment of adrenal vein cannulation. Use of 18OHDOC-derived SI eradicates the need for ACTH stimulation during AVS. LIs based on F, B, DOC, and 18OHDOC exhibit comparable identification of PA subtype.

MICHAEL PAYNE

Supervisor: Dr. Diane Roscoe
Session: Clinical Sciences

"Evaluation of two chromogenic media for the isolation and identification of urinary tract pathogens (UTP)"

AUTHORS:
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Background/objectives:
Chromogenic media (CM) are available for urine specimens (US) to enable rapid identification of common pathogens. Two CM, chromID CPS 4(bioMérieux) and UriSelect (URS) 4 (Bio-Rad) were compared to the standard media (SM) used for US.

Methods:
The performance of CM was compared to a routine protocol (BAP and MAC) for the isolation and identification of UTP. In June/July 2012, all US received between 9am-5pm on 10 specific days were inoculated to CPS 4, URS 4, BAP and MAC plates using 1µL and 10µL for non-invasive and invasively collected US, respectively. CM interpretation was done according to the product inserts by one person blinded to the results of SM. SM were read by experienced technologists according to protocol and isolates were identified using BD Phoenix™. Results were grouped into significant (SG), mixed (MG), and no significant growth (NSG). SG was defined as $\geq 10^7$ CFU/L, MG as ≥ 3 species, and NSG as $<10^7$ CFU/L for standard specimens.

Results:
903 US were studied. SM identified 239 SG, 112 MG, and 552 NSG cultures. The most common pathogens were E.*coli* (38%) and *Enterococcus* spp. (11%). Comparing CM to SM, the exact agreement (same species isolated) was 89.3% and 89.5% for URS 4 and CPS 4, respectively. When grouped by clinical significance (MG, NSG, and SG-same species), agreement with SM was 93.0% and 93.1% for URS 4 and CPS 4, respectively. CM were equivalent with respect to processing time, both required less than SM.

Conclusions:
Both CM compared well to SM and allowed for rapid preliminary identification of many UTP. Advantages include decreased labour and need for other identification of certain species, particularly E.*coli*. In terms of workflow, CM enables same-day identification for almost 50% of significant UTP.

AMANDA WILMER

Supervisor: Dr. Mark Hull
Session: Clinical Sciences

"*Shigella flexneri* serotype 1 infections in men who have sex with men in Vancouver, Canada"

AUTHORS:
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Background/objectives:
Shigella outbreaks in men who have sex with men (MSM) are described periodically, presumably related to sexual practices in this population. Recently, outbreaks of Shigella flexneri serotype 3 have been described in the United Kingdom and United States. We observed a rise in Shigella flexneri isolates and related admissions at our hospital, and hypothesized that increased local transmission may be occurring.

Methods:
We performed a retrospective review of S. flexneri cases identified by the microbiology laboratory of a tertiary care centre in Vancouver, Canada, from August 2008-2012. Available hospital charts were reviewed for additional clinical and laboratory information. The majority (67.1%) of isolates were sent to a reference laboratory for serotyping and pulsed-field gel electrophoresis (PFGE).

Results:
Seventy-six cases of *Shigella flexneri* enterocolitis were diagnosed in 71 male patients, with a median age of 44 years (interquartile range (IQR) 37.5 to 50 years). In total, 85.9% (61/71) were infected with human immunodeficiency virus (HIV). Sexual orientation was noted in 58/71 (81.7%) cases, with all 58 noted to be MSM. Sixty-six (93.0%) cases had a single episode of Shigellosis, while 5 (7%) cases had 2 episodes. Travel history was available in 46/76 (60.5%) cases, 2 of which had traveled to Mexico. Blood cultures were positive in 3/29 (10.3%) cases tested. Forty-six (60.5%) cases presented to the emergency department, while 29 (38.2%) were hospitalized a median of 3 days (IQR 2-6 days). Twenty-seven of 29 (93.1%) hospitalized patients were HIV positive. Forty-three (65.2%) HIV positive cases had an undetectable viral load (VL), with a median CD4 count of 470/mm3 (IQR 375-630) while the remaining 23 had a median VL of 7686 copies/mL (IQR 52-67610 copies/mL), with a median CD4 count of 370/mm3 (IQR 70-485/mm3). Overall, 51/76 (67.1%) S. flexneri isolates underwent serotyping, with 46/51 (90.2%) found to be Type 1 and 5/51 (9.8%) Type 3. Of the Type 1 isolates, 32/46 (69.6%) had indistinguishable PFGE patterns (SFXXAI.0076).

Conclusions:
We identified ongoing transmission of *Shigella flexneri* Type 1 in our local MSM population, in whom HIV was prevalent. Although the majority of cases appeared to have well controlled HIV, more than a third of cases required hospitalization, including 3 cases with bloodstream infection. Educational interventions targeted toward this population.

Resident | Oral Session

VERONICA HIRSCH-REINSHAGEN

Supervisor: Dr. Ian Mackenzie
Session: Clinical Sciences

"Clinicopathological correlations in frontotemporal dementia and amyotrophic lateral sclerosis due to C9orf72 repeat expansion"

AUTHORS:
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Recently discovered expanded GGGGCC hexanucleotide repeats in a non-coding region of the chromosome 9 open reading frame 72 gene (C9orf72) cause an autosomal-dominant familial form of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), where affected members develop either FTD or ALS or both. In addition, these repeats are the most common genetic abnormalilty underlying familial and sporadic forms of both FTD and ALS. The neuropathology associated with this mutation is characterized by abnormal accumulation of the transactive response DNA binding protein with M 43kD (TDP-43) in neurons and glia. An even more specific feature is the presence of additional neuronal inclusions that are immunoreactive for markers of the ubiquitin proteosome system, including ubiquitin and p62, but negative for TDP-43. In this study we evaluated clinicopathological correlations in patients with C9orf72 repeat expansion.

Postmortem examination of the brain and spinal cord was performed in 24 patients carrying the C9orf72 repeat expansion. Clinical diagnoses of FTD and ALS were based on the Neary and El Escorial criteria, respectively. Histological stains and immunohistochemistry were performed on formalin fixed, paraffin-embedded tissue sections using established techniques. Multiple brain and spinal cord regions were evaluated for TDP-43, ubiquitin and p62 immunoreactive pathology and graded semiquantitatively (0, absent; 1+, rare; 2+, mild; 3+, moderate; 4+, severe). Statistical analyses were performed using non-parametric tests.

TDP-43 positive neuronal pathology was widely distributed and involved neocortex, limbic system, basal ganglia, brainstem, cerebellum and spinal cord. The degree of TDP pathology was found to correlate with clinical phenotype; cases with a predominant FTD phenotype had a high burden of TDP-43 positive neuronal inclusions in frontal cortex and hippocampus, whereas patients with a predominant ALS phenotype had extensive spinal cord involvement. However, patients with FTD phenotype also had some degree of spinal cord involvement and ALS patients always showed some neocortical and limbic TDP pathology. In contrast, ubiquitin and p62 positive inclusions were usually present in high numbers in all examined areas and did not correlate with the clinical phenotype. In addition, a striking disconnect between TDP and ubiquitin/p62 pathology was observed in several neuroanatomical regions, where TDP pathology was minimal or absent even though ubiquitin/p62 inclusions were abundant.

In summary, the clinical phenotype in cases of FTD/ALS due to C9orf72 repeat expansion correlates with the distribution and severity of TDP-43, but not ubiquitin/p62, immunoreactive pathology. Involvement of both cerebral cortex and pyramidal motor system in all subjects is in keeping with the notion that all patients with the C9orf72 mutation are at risk of developing both FTD and ALS. The consistent finding of numerous ubiquitin/p62 positive, TDP-43 negative neuronal inclusions in many neuroanatomical regions suggests that this additional pathology is either not directly involved in the pathogenesis of disease or that it is a generalized phenomenon that triggers the disease-related TDP pathology in susceptible neuronal populations.

PETER SCHUTZ

Supervisor: Project supervisor Dr. Stephen Yip
Session: Clinical Sciences

"Suprasellar hemangioblastoma during pregnancy – a case analysis"

Background/objectives:
Hemangioblastoma in supratentorial location is rare and shows strong association with von Hippel Lindau disease and considerable variability of observed growth patterns. Little is known about determinants of its biological behavior.

Methods:
Single case analysis of unresectable supratentorial hemangioblastoma diagnosed during pregnancy and treated by external beam radiation.

Results:
A 30-year-old pregnant female presented at 35 weeks gestation with dramatically deteriorating vision and personality changes. MRI showed a 3.6 x 3.6 x 3 cm, suprasellar, uniformly enhancing mass. To facilitate neurosurgical intervention, the baby was delivered by Cesarean section. However, excessive bleeding during tumour surgery precluded resection. Biopsy showed a low grade, inhibin-positive clear cell neoplasm diagnostic of hemangioblastoma. Its suprasellar location suggested von Hippel Lindau disease. Post-operatively, the patient was bilaterally blind with mild vision improvement during the immediate postoperative period. She underwent fractionated external beam radiation therapy. Five months after surgery, vision was considerably improved to almost normal with radiologically confirmed tumour shrinkage.

Conclusions:
This case demonstrates rapid growth of supratentorial hemangioblastoma during preganancy with impressive tumor shrinkage post partum and radiation therapy, suggestive of endocrine/angiogenic factors as possible determinant of tumour growth. Pathology of hemangioblastoma including effects of pregnancy and radiation therapy are reviewed.

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Resident | Oral Session

MAXIM SIGNAEVSKI

Supervisor: Dr. Cyril Blake Gilks and Dr. Stephen Yip
Session: Clinical Sciences

"IDH1 R132H immunohistochemical staining in laboratories across Canada showed accurate and reproducible results"

AUTHORS:
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Background/objectives:
Specific mutations in the isocitrate dehydrogenase gene IDH1 have been found in glial tumors, with mutations found in nearly all cases of secondary glioblastomas, which develop from lower-grade gliomas, but rarely in primary high-grade glioblastoma multiforme. Presence of IDH1 mutation in tumor is shown to correlate with longer survival. IDH1 R132H mutation IHC is becoming more and more widely used in brain tumor diagnosis; however, not every pathology laboratory uses it routinely.

Methods:
We have constructed a TMA with known 13 IDH1 R132H-positive and 17 IDH1 R132H-negative gliomas. The IDH1 R132H immunostaining was performed using local protocols at six laboratories across Canada. This project was undertaken as part of the national Canadian Immunohistochemistry Quality Control (CIQC) effort to provide External Quality Assurance (EQA) to ensure high quality immunostaining in clinical pathology laboratories.

Results:
There were 29-30 interpretable TMA cores from each laboratory. The quality of staining was good to excellent with little to no background. All cores showed IHC staining that was consistent with both genetic test results and with IHC in other laboratories. We have conducted blinded validation of IDH1 R132H IHC from different laboratories across Canada and achieved a 100% concordance in our calls.

Conclusions:
The TMA validation and IHC protocol comparison showed that despite protocol variability between laboratories, IDH1 R132H IHC is a reliable, accurate and reproducible technique for routine diagnostic practice.

KEVIN YANG

Supervisor: Dr. Marianne Sadar
Session: Basic Sciences

"The battle is on: small molecule EPI-002 versus aberrant androgen receptor activity in castration-resistant prostate cancer"

AUTHORS:
Yu Chi (Kevin) Yang[1], Nasrin (Rina) Mawji[1], and Marianne Sadar1[1]

AFFILIATIONS:
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Background/objectives:
The incidence and mortality rates of prostate cancer (PC) remain high among North American men, and there is no cure for advanced PC. Androgen deprivation therapy (ADT) has remained the mainstay treatment for advanced PC. Currently, all approaches to ADT target the C-terminal ligand-binding domain (LBD) of androgen receptor (AR), but unfortunately these therapies, including castration and antiandrogens, will eventually fail as patients develop lethal castration-resistant prostate cancer (CRPC). Compelling evidence indicates that the AR, a transcription factor of the steroid hormone receptor family, remains functionally active and plays a critical role in PC. AR regulates genes that are responsible for the growth and survival in PC cells, and persistent and aberrant AR transcriptional activity has been suggested to be a major mechanism underlying CRPC. The N-terminal domain (NTD) of AR is essential for its activity and has been validated as a viable therapeutic target for PC. Here, the objectives are to investigate if EPI-002, an inhibitor of the AR NTD, can overcome molecular alterations underlying aberrant AR activity in CRPC, such as the overexpression of AR co-activators and the presence of constitutively active AR splice variants.

Methods:
LNCaP human PC cells were transiently transfected with an AR-driven luciferase reporter gene and various amount of expression vector for AR co-activator SRC3. These cells were treated with DMSO or EPI-002 in the presence or absence of androgen. 24 hours after treatment, the cells were harvested for luciferase assay to measure AR transcriptional activities. LNCaP95 cells, derived from LNCaP cells cultured under prolonged androgen-depletion, express constitutively active AR splice variant V7 (AR-V7) in addition to full-length AR. AR transcriptional activities were examined in LNCaP95 cells treated with EPI-002 or antiandrogen by QPCR for the endogenous mRNA expression of several genes that have been reported to be regulated by AR-V7. Proliferation of LNCaP95 cells treated with DMSO, EPI-002, or antiandrogens was measured using BrdU incorporation that quantifies the rate of DNA synthesis.

Results:
Overexpression of AR co-activator SRC3 led to increased AR transcriptional activity in LNCaP cells as expected, but EPI-002 maintained its strong inhibition on AR activity regardless of the elevated SRC3 expression. LBD-truncated AR splice variant V7 is constitutively active and can drive CRPC tumor growth in mice. Recently, AR-V7 has been shown to mediate a distinct transcriptome from that of the full-length AR. Here we demonstrated for the first time that EPI-002 significantly decreased the mRNA expression levels of several genes regulated by AR-V7 in LNCaP95 cells, whereas LBD-targeting antiandrogen had no effect on the expression of these genes. Consistent with the QPCR data, EPI-002 significantly inhibited the androgen-independent and antiandrogen-resistant proliferation of LNCaP95 cells.

Conclusions:
EPI-002 is able to overcome two clinically relevant molecular alterations that contribute to aberrant AR activity in CRPC: AR co-activator overexpression and constitutively active AR splice variant. Our findings demonstrated the therapeutic benefits of AR NTD inhibitors in the treatment of CRPC.

Graduate Student | Oral Session

BRYANT HARBOURNE

Supervisor: Dr. Kevin Bennewith
Session: Basic Sciences

"Hypoxia induced secreted proteins and the pre-metastatic niche"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Metastatic cancer is responsible for 90% of cancer related deaths. Past research has focused mainly on the primary tumour leaving the process of metastasis poorly understood. Recently, the "pre-metastatic niche" hypothesis of metastasis has gained acceptance. The pre-metastatic niche represents an area in metastatic target organs where the extra cellular matrix has been remodelled by tumour secreted proteins and there has been an accumulation of bone marrow derived cells. We believe that these bone marrow cells are immature myeloid cells and myeloid derived suppressor cells (MDSC's). Together the architectural change in extra cellular matrix and the presence of immunosuppressive myeloid cells creates an area permissive to the invasion, survival and growth of circulating tumour cells. It is known that poorly oxygenated (hypoxic) tumour cells have a more aggressive and invasive phenotype which correlates with poorer prognosis. Poorly functional tumour blood vessels cause a fraction of the tumour to become hypoxic and express hypoxia inducible proteins.They are responsible for increased angiogenesis, invasion, matrix deposition and remodelling along with many other functions. We hypothesize that hypoxic tumours produce and secrete proteins distinct or at increased levels from normoxic tumours and that those secreted proteins are required for pre-metastatic niche development. Our aim is to identify the hypoxia induced secreted protein(s) responsible for the architectural changes in metastatic organs related to pre-metastatic niche development.

Methods:
We utilized Stable Isotope Labelling of Amino acids in Cell culture (SILAC) to perform a quantitative proteomic screen of conditioned medium. Mammary carcinoma cells derived from a Balb/c mouse (4T1 - metastatic tumour cell line and 67NR - non-metastatic tumour cell line) were placed in 1% O2 (hypoxic) or 21% O2 (normoxic) for 24 hours. The secreted proteins were collected and subjected to mass spectrometry (LC/MS-MS). Using lentiviral transduction of short hairpin RNA (shRNA) we knocked down candidate proteins identified in the proteomic screen and examined the phenotypic effect of the 4T1 tumour cells. *In vitro*, the invasion and migration capabilities will be studied using Boyden chamber assay. In vivo, the knock down cells will be orthotopically implanted in syngeneic Balb/c mice. Metastasis can be assessed using flow cytometry-based quantification of tumour cells in the mouse lungs. In the future TNC will be over-expressed in the 4T1 and 67NR tumour cell lines and the effect of TNC over-expression will be examined *in vitro* and *in vivo*.

Results:
The proteomic data allowed selection of Tenascin C (TNC) as a candidate secreted protein with a role in pre-metastatic niche development. Knock down of TNC had no effect on the invasive ability of 4T1 tumour cells in vitro. *In vivo*, knock down of TNC in 4T1 tumour cells did not affect primary tumour growth but did result in a significant decrease in 4T1 tumour cells present in the lung indicating reduced lung metastasis.

Conclusions:
TNC has a role in pre-metastatic niche formation and loss of TNC impairs the ability of 4T1 tumour cells to metastasize to the lungs. TNC represents a potential new therapeutic drug target.

ADA KIM

Supervisor: Dr. Kevin L. Bennewith
Session: Basic Sciences

"The role of heme oxygenase-1 (HMOX1) in breast cancer metastasis"

AUTHORS:
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Background/objectives:
The metastatic spread of cancer is linked to over 90% of cancer-related deaths. Therapies designed to prevent the dissemination of metastatic cells from the primary tumour hold great promise to improve outcome for patients at risk of developing metastatic disease. Heme oxygenase-1 (HMOX1) is the rate-limiting enzyme in heme catabolism and is induced by various stress stimuli including reactive oxygen species, heat shock, and hypoxia (low oxygen levels). HMOX1 is a well-characterized and studied enzyme, yet there are inconsistencies in terms of its role and expression in different cancer types. Specifically, the role of HMOX1 in breast cancer metastasis is controversial with some groups indicating that HMOX1 promotes metastasis while others indicate HMOX1 reduces metastatic spread. We have previously shown that inducing expression of HMOX1 by treatment with hemin (a chemical inducer of HMOX1) decreased tumour cell migration and invasion *in vitro*. We hypothesize that HMOX1 reduces breast cancer metastasis by decreasing tumour cell migration and invasion. We are interested in examining whether HMOX1 reduces breast cancer metastasis and therefore represents a potential therapeutic adjuvant for patients with metastatic disease.

Methods:
We used three different murine mammary carcinoma cell lines as models for breast cancer: 67NR - noninvasive and not metastatic, 4TO7 - invasive, and metastatic, 4T1 - highly aggressive metastatic behavior. These cell lines were genetically modified to overexpress HMOX1 or with HMOX1 knocked down. We assessed migration and invasion *in vitro* by conducting transwell Boyden chamber assays. In addition, the genetically modified cells were orthotopically implanted in BALB/c mice to study primary tumour growth and metastasis *in vivo*.

Results:
The results from inducing HMOX1 with hemin were validated by using CoPP, another chemical inducer of HMOX1, as well as genetic overexpression of HMOX1 in *in vitro* migration and invasion assays. Both CoPP and the genetic overexpression similarly resulted in the reduction of migration and invasion of the tumour cells. When 4T1 cells overexpressing HMOX1 were implanted in BALB/c mice, there was no difference in primary tumour growth or lung metastases. However, 4T1 cells with HMOX1 knocked down resulted in an increase in lung metastases *in vivo*.

Conclusions:
HMOX1 expression decreases the migration and invasion of murine mammary carcinoma cells *in vitro*, while knockdown of HMOX1 increases metastasis *in vivo*. Potential interactions between HMOX1 and ECM proteins will be examined to study the mechanism of HMOX1-mediated decreases in metastasis. Our data indicate the importance of HMOX1 in breast cancer metastasis and suggest that HMOX1 may represent a therapeutic adjuvant for metastatic breast cancer.

MOMIR BOSILJCIC

Supervisor: Dr. Kevin Bennewith
Session: Basic Sciences

"Myeloid-derived suppressor cell accumulation in secondary target organs is tumour hypoxia driven and promotes a higher metastatic potential in breast cancer"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Solid tumours contain cells that are poorly oxygenated (hypoxic). Not only is it harder to kill hypoxic cells with conventional therapies, but they also promote a more metastatic tumour phenotype. The metastatic spread of cancer is associated with over 90% of cancer-related deaths. It has been shown that proteins secreted by tumours can stimulate the accumulation of bone marrow-derived cells (BMDCs) in tissues prior to metastatic tumour cell arrival. BMDC accumulation may enhance the survival and growth of metastatic tumour cells, and predict future sites of metastatic tumour growth. Myeloid derived suppressor cells (MDSCs) are a type of BMDC, classified by their ability to suppress T-cell mediated immune responses. In doing so, MDSCs enable tumour cells to survive and proliferate to form secondary metastatic tumours. While tumor hypoxia and MDSC accumulation in metastatic target organs have been linked to poorer patient outcome, their interplay in metastatic progression remains unclear. In our animal model, we have found that tumour hypoxia is required for MDSC induction and accumulation in the spleen and lungs of Balb/c mice. The objective of the study was to determine whether targeting hypoxic tumour cells will decrease MDSC accumulation in secondary tissues and if depletion of MDSCs will decrease metastatic tumour growth.

Methods:
Primary tumours and lung sections of mammary cell lines with different inherent metastatic potentials (4T1-highly metastatic, 4T07-moderately metastatic, 67NR-non-metastatic, PyMT-spontaneous metastatic) were immunofluorescently (IF) stained to illustrate the extent of their hypoxia or MDSC accumulation. We utilized tirapazamine (TPZ) and gemcitabine (GEM) as tools to target hypoxic tumour cells and MDSCs respectively in the highly metastatic 4T1 model. MDSC (CD11b+Gr1+) and hypoxic tumour cell populations (Pimonidazole positive) were analyzed by flow cytometry. Additionally, we found that elevated levels of MDSCs persist in the lungs even after tumour resection. As a result, we plan to utilize GEM to target the persistent MDSCs and gain insight into their role in promoting secondary tumour re-growth.

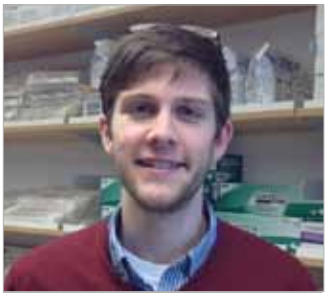
Results:
Our IF staining shows that MDSC accumulation in metastatic target organs positively correlates with tumour aggressiveness (metastatic spread). We show that a tumour needs to be hypoxic in order to recruit MDSCs from the bone marrow. The recruited MDSCs remain in the lungs after tumour resection and promote metastatic tumour growth. Treatment with TPZ (targets hypoxic cells) reduces the number of MDSCs in spleens and lungs of 4T1 tumour bearing mice without affecting primary tumour growth kinetics. We show that GEM effectively depletes MDSC in 4T1 tumour bearing mice without affecting tumour growth.

Conclusions:
Improving our understanding of the development of metastatic tumours is central to effectively treating tumour metastases. Our research provides valuable insight into the role of hypoxic tumour cells and MDSCs in promoting metastatic tumour growth and will aid in the design of improved treatments for patients with metastatic cancer.

DAVID TWA

Supervisor: Dr. Christian Steidl
Session: Basic Sciences

"Tumour microenvironment-altering genomic rearrangements in B-cell lymphomas"



AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Lymphoid cancers are among the most prevalent malignancies in Canada and regularly manifest in younger populations. There is increasing evidence that the tumour microenvironment plays a critical role in the pathogenesis and clinical behaviour of lymphomas, including Hodgkin lymphoma (HL), primary mediastinal B-cell lymphoma (PMBCL) and subsets of diffuse large B-cell lymphoma (DLBCL). Analysing these cancers with high-throughput sequencing techniques, we recently discovered recurrent structural changes in the genes *CD274* (PDL1) and *PDCD1LG2* (PDL2), which led to altered transcript levels. Increased expression of these genes is known to dynamically alter the tumour microenvironment in favour of malignant cell survival and proliferation. This is achieved by actively and passively repressing the activity and infiltration of tumour-targeting immune cells. We report frequencies, transcript and protein expression levels, and translocation partners of programmed death ligand PDL rearrangements in a large extension cohort of B-cell lymphomas.

Methods:
We determined the frequencies of copy number changes and chromosomal rearrangements in clinical cases of PMBCL, immune-privilege DLBCL and established B-cell lymphoma cell lines by fluorescence *in-situ* hybridization (FISH). Genomic breakpoints for PDL1 and 2 (9p24.1) were identified in cases with an available source of fresh-frozen DNA and RNA by means of long-distance inverse (LDI)-PCR or whole genome shotgun sequencing (WGSS). Transcriptomic fusion partners were identified by whole transcriptome shotgun sequencing (WTSS) or rapid amplification of cDNA ends (RACE). PDL transcript expression levels in cell lines were established using quantitative reverse transcriptase (qRT)-PCR. Surface PDL expression was determined using four-channel flow cytometry.

Results:
Screening over 500 B-cell lymphoma samples identified a PDL locus aberration rate of between 80% (PMBCL) and 15% (DLBCL). Identified aberration partners included: *CIITA*, *RFX3*, *NRG1*, *IGHV7-81*, *IGHG,* and *LRMP*. We also found an interstitial deletion in the nodular lymphocyte-predominant (NLP) HL cell line DEV, producing a novel RELB-CLASRP fusion that might synergize with the other CIITA-PDL2 gene fusion also found in this cell line. Characterization of genomic breakpoints revealed two cluster breakpoint regions producing two types of gene fusions with PDL either at the 5' or 3' end of the chimeric mRNA. HL cell lines were found to have higher levels of PDL1 transcript and protein expression in comparison to NLPHL and PMBCL cells lines, while L-1236 (an HL cell line) and all DLBCL cell lines showed little PDL protein expression.

Conclusions:
We have identified novel, recurrent rearrangements of the PDL locus in B-cell lymphomas. This finding has implications for the classification and diagnoses of B-cell lymphomas. Our data also indicates that PDL locus rearrangement or amplification leads to over-expression of PDL1 and PDL2. With these observations in mind, ongoing studies will characterize PDLs as a personalized therapeutic target.

Graduate Student | Oral Session

JESSE OLSON

Supervisor: Dr. Angela M. Devlin
Session: Basic Sciences

"Programming of glucose homeostasis by gestational exposure to folic acid and vitamin B12 imbalance"

AUTHORS:
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Background/objectives:
Maternal nutritional status during pregnancy influences offspring health in adulthood by programming metabolism. This is the phenomenon of, "developmental programming". The mechanisms underlying developmental programming are unknown but may involve epigenetic processes, such as DNA methylation. The synthetic folate, folic acid (FA), and vitamin B12 play crucial roles in providing methyl groups for methylation reactions. A recent longitudinal study from India reported that children born to women with adequate folate and low B12 status during pregnancy had greater adiposity and insulin resistance at the age of 6, which are risk factors for developing type 2 diabetes mellitus and cardiovascular disease. These findings are particularly relevant to countries, including Canada, which fortify their grain products with FA. In Canada as many as 1 in 20 women may be B12 deficient in the early stages of pregnancy. Currently, the metabolic consequences of elevated folic acid with or without B12 deficiency during pregnancy are unknown. The objective of this study is to determine if a maternal diet deficient in B12 and high in folic acid programs glucose homeostasis and adiposity in female mice at 20 and 30 weeks of age.

Methods:
Female mice were fed a control diet, FA=2 mg/kg; B12=50 µg/kg; high FA no B12 diet (HFA-B12), FA=10 mg/kg; or a high FA with B12 (HFA+B12) diet, FA=10 mg/kg; B12= 50 µg/kg. Offspring n=12/diet) were weaned onto the control diet, or a western diet (45% fat, excess energy) to determine the effect of maternal diet on offspring response to a post weaning obesogenic diet. Glucose tolerance was assessed after 20 and 30 weeks of feeding in fasted mice. A 25% dextrose solution (dose of 0.75/kg body mass) was administered via intraperitoneal injection and blood glucose levels were measured over a two hour period. Fasting insulin, serum total adiponectin and high molecular weight adiponectin were measured using ultra sensitive ELISAs. At 30 weeks body composition was determined by quantitative magnetic resonance and adipose depots were measured during surgical dissection.

Results:
At 20 weeks of age, HFA-B12/control fed mice showed impaired glucose tolerance compared to control and HFA+B12 fed mice ($P<0.05$) while no differences were observed in western fed mice. Basal glucose concentrations were similar in all diet groups whereas basal insulin levels were increased two fold in mice fed a western weaning diet compared to control weaned mice ($P<0.05$). At 30 weeks of age, mice fed a western weaning diet demonstrated greater impaired glucose tolerance than control diet weaned mice ($P<0.05$). Basal glucose ($P<0.05$) and insulin ($P<0.05$) levels were greater in western diet weaned mice compared to control weaned mice while serum total and high molecular weight adiponectin were unaffected by diet. HFA-B12/western mice had lower basal glucose concentrations compared to control/ western mice ($P<0.05$). Basal insulin levels were decreased in HFA-B12/western mice compared to control/western ($P<0.05$) and HFA+B12/western mice ($P<0.05$). Percent fat mass was increased in western fed mice ($P<0.0001$) while fat depots were unaffected by maternal FA/B12 status.

Conclusions:
This data suggest that exposure to imbalanced FA and B12 intake during gestation programs glucose homeostasis in mice. Further work is required to uncover the mechanisms responsible for this programming effect.

GABRIEL FUNG

Supervisor: Dr. Honglin Luo
Session: Basic Sciences

"Coxsackievirus B3 disrupts cytosolic stress granules by viral protease 3C"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Coxsackievirus type 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. In response to viral-induced stress, dynamic cytosolic aggregates called stress granules (SGs) accumulate, consisting of proteins and mRNA. A key component of SGs is Ras-GAP SH3 domain binding protein-1 (G3BP1), which in part mediates protein-protein and protein-RNA interactions. SGs are critical in mRNA storage and metabolism during stress, and have been implicated in several human diseases, viral infection, inflammation, cancer and many neurological diseases. In this study, we aim to elucidate the mechanism and significance of SGs in CVB3 infection. **We hypothesise that CVB3-induced stress granule formation plays a protective role against CVB3 infection**.

Methods:
HeLa cells stably expressing green fluorescent protein (GFP-) tagged G3BP1 were used to monitor the formation of SGs during CVB3 infection. To explore the potential mechanism by which CVB3 regulates SG formation, we examined protein expression of G3BP1 and G3BP1Q325E, a point mutation mutant of G3BP1, in CVB3-infected cells by confocal microscopy and immuno-electron micrscopy. Finally, RT-PCR, immunoblot and plaque assay were used to investigate the significance of G3BP1 overexpression and siRNA knockdown in HeLa cells.

Results:
Confocal imaging showed punctate accumulation of GFP-G3BP1 fluorescence in the cytosol at ~3 hours post infection (hpi) and disappearance ~5 hpi, while GFP-G3BP1Q325E mutants retained SG formation throughout CVB3 infection. Immuno-EM showed G3BP1-positive SGs localized near mitochondrial surfaces. By immunoblot, we showed that G3BP1 was cleaved at 5 hpi by viral protease 3C. Finally by RT-PCR, immunoblot and plaque assay, viral replication was significantly reduced in G3BP1 overexpressing cells compared with wild-type HeLa cells. Moreover, knockdown of G3BP1 resulted in increased viral replication.

Conclusions:
SG formation occurs during the early phase of CVB3 infection, however at late stages of viral infection, disassembly occurs due to 3C-mediated G3BP1 cleavage at amino acid Q325. Our data suggest a protective role of G3BP1-SGs during CVB3 infection. Results generated from this study may potentially aid in developing novel therapeutics against CVB-induced diseases.

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

ADA LEUNG

Supervisor: Dr. Marcel Bally
Session: Basic Sciences

"Silencing of PAPSS1 (3'-phosphoadenosine 5'-phosphosulfate synthase 1) potentiates cisplatin activity against non-small cell lung cancer "

AUTHORS:
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Background/objectives:
Non-small cell lung cancer (NSCLC) accounts for more than 80% of all lung cancers with two out of three patients having an inoperable disease at the time of diagnosis. Standard first-line treatments for these patients are combinations of two chemotherapy drugs, with one typically being cisplatin. Despite the use of new targeted therapies and attempts to develop triplet combinations, the overall 5-year survival rate for NSCLC remains below 20%. A genome-wide siRNA (small-interfering RNA) screen was performed in A549 cells to rapidly identify individual genes that, when silenced, would enhance cisplatin activity. One of the top targets that emerged from the screen was 3'-phosphoadenosine 5'-phosphosulfate (PAPS) synthase 1 (PAPSS1), a bifunctional enzyme that synthesizes PAPS, the universal sulfate donor. Here, we evaluated PAPSS1 as a cisplatin-potentiating gene target in NSCLC.

Methods:
The effect of PAPSS1 inhibition on cisplatin activity was assessed across eight different chemotherapy-naïve NSCLC cell lines. Cells were transfected with lipid-complexed siRNA targeting PAPSS1 and treated with various concentrations of cisplatin 24 hours later. The dose response was determined based on viable cell count 72 hours post-treatment using the IN Cell Analyzer. Gene knockdown was confirmed via qPCR and Western blot analysis.

Results:
Silencing of PAPSS1 resulted in significant reduction in the IC50 of cisplatin in four chemotherapy-naïve NSCLC cell lines at siRNA concentrations that would not induce significant lipid toxicity over a 72-hour culture period. For example, PAPSS1 silencing was associated with a 5-fold decrease ($p<0.001$) in the cisplatin IC50 in A549 cells. No significant toxicity or enhancement in cisplatin activity was observed when PAPSS1 was knocked down in primary epithelial cells of the airway. Preliminary studies performed with sodium chlorate, a known non-selective PAPSS1 inhibitor, yielded a 1.5-fold decrease in the IC50 of cisplatin in A549 cells. We have also demonstrated that the extent of cisplatin potentiation positively correlates with the level of PAPSS1 inhibition at the protein level.

Conclusions:
Our results highlight the need for more potent PAPSS1 inhibitors to further investigate the feasibility of developing PAPSS1 as a cisplatin-potentiating therapeutic target. Furthermore, these results identify PAPSS1 as a novel cisplatin-potentiating target in NSCLC and also validates a drug combination discovery program designed to identify genes that when silenced/inhibited, enhance the action of standard of care cytotoxic agents used against chemotherapy-naïve cancers.

MELISSA MCCONECHY

Supervisor: Dr. David Huntsman
Session: Basic Sciences

"Intratumoural heterogeneity and the detection of plasma circulating tumour DNA in endometrial carcinomas"

AUTHORS:
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Background/objectives:
Endometrial carcinoma is the most common gyneacological cancer found in North American women. Approximately 20% of endometrial carcinomas are high-grade tumours and are associated with poor pathologic diagnostic reproducibility. Recent molecular studies are associating mutations with specific endometrial subtypes to improve diagnostic reproducibility. Intratumoural heterogeneity has been shown to be present in multiple tumour types (lung, breast, ovary) where there are differences in mutations between areas of primary and metastatic tumours, however this has not been fully elucidated in endometrial carcinoma. In addition, the use of plasma circulating tumour DNA (ctDNA) has been found to be a non-invasive diagnostic tool in many cancer types. The potential use of detecting mutations in ctDNA from endometrial cancer patients may be beneficial for diagnosis and disease monitoring over time. The objective of this study is to determine if intratumoural heterogeneity is present in endometrial carcinoma, and if mutations identified in the primary tumour samplings can also be detected in plasma ctDNA.

Methods:
Two endometrial carcinoma cases were identified from VGH, and DNA was harvested from six different tumour sites within the same primary tumour. Normal DNA was extracted from blood buffy coat and plasma was used to isolate ctDNA. A custom gene panel was designed using the Illumina DesignStudio software to interrogate approximately 1500 amplicons. The Illumina TruSeq library preparation kits were used to prepare sequencing libraries. Samples were indexed, pooled, and sequenced on the Illumina MiSeq using 300 cycle reagent kits.

Results:
Within the first endometrial case, mutations in PTEN, PIK3CA, CTNNB1, ARID1A, CHD4 and CTCF were identified within the primary tumour. ARID1A and CTNNB1 mutations showed differences in the frequency and presence of mutations within the different tumour samplings. Mutations identified in PIK3CA and CTCF were also present in the ctDNA at low frequencies. Mutations in PIK3CA, TP53, and CHD4 were detected in the second endometrial case in all samplings of the tumour. One low frequency POLE mutation was identified in only two tumour samplings. The PIK3CA and CHD4 mutations found in the primary tumour were also identified at high frequencies within the plasma ctDNA of this patient.

Conclusions:
Sequencing multiple samplings of endometrial carcinoma cases shows that there is variation in the mutations present in different areas of the same tumour. This indicates that intratumoural heterogeneity is present, and may play a role in the poor pathologic diagnostic reproducibility of this tumour type. We have also shown that the detection of ctDNA mutations within endometrial carcinomas is feasible and may be useful in primary diagnosis and used for monitoring disease over time.

Graduate Student | Oral Session

ERICA OSBOURNE

Supervisor: Dr. Marianne D. Sadar
Session: Basic Sciences

"Small molecule EPI-002 inhibits transcriptional activities of androgen receptor and truncated variants in castrate-resistant prostate cancer"

AUTHORS:
Erica K. Osbourne, Nasrin R. Mawji, Marianne D. Sadar

AFFILIATIONS:
Michael Genome Sciences Centre, BC Cancer Agency

Background/objectives:
The growth and progression of prostate cancer is dependent on the transcriptional activity of the androgen receptor (AR). Full-length AR (fl-AR) is structurally composed of an androgen-independent transactivation amino-terminal domain (NTD), a DNA-binding domain and an androgen-dependent ligand-binding domain (LBD). The NTD harbors all transcriptional activity and is required for AR function. Current androgen deprivation therapies and antiandrogens, such as MDV3100, targeted against AR LBD are initially effective in men with recurrent prostate cancer. However, the response is transient and eventually all men will progress to lethal castration-resistant prostate cancer (CRPC) due to resistance developed by AR adaptation mechanisms. One potential mechanism is alternative splicing of AR that forms constitutively active truncated AR variants that lack the full LBD. AR V7 and V567es are the most prominent and clinically relevant AR variants correlated with poor prognosis in patients with CRPC. Since current therapies target the LBD, which is not present in these AR variants, a novel NTD small molecule inhibitor that blocks transcriptional activity of variants and fl-AR would significantly improve the clinical management of CRPC. Small molecule EPI-001 is a first-in-class AR NTD inhibitor composed of four stereoisomers. EPI-002 is the most active stereoisomer of EPI-001. By inhibiting NTD transcriptional activity of all AR species, EPI compounds will be assessed as potential therapeutic agents for patients presenting with tumours expressing mixed populations of fl-AR and AR variants. We hypothesize that: (1) NTD antagonist EPI-002 inhibits transcriptional activity of endogenous and exogenous AR V7 in human prostate cancer cell lines, and (2) combination treatment of EPI-002 and MDV3100 demonstrates greater inhibition of transcriptional activity of mixed populations of both fl-AR and truncated splice variants than each treatment alone.

Methods:
Human prostate cancer PC3 cells that do not express functional AR were transiently co-transfected with an expression plasmid for AR V7 with AR-driven firefly-luciferase reporter gene constructs and treated with EPI-002 or DMSO as control. Endogenous fl-AR expressing LNCaP human prostate cancer cells were transfected with AR V7 expression plasmid and AR-driven firefly-luciferase reporter genes then treated with EPI-002, MDV3100 or DMSO as control in the presence and absence of synthetic androgen R1881. Transcriptional activity was measured using firefly luciferase assay system. Levels of endogenous and ectopic AR protein were determined by Western Blot analysis.

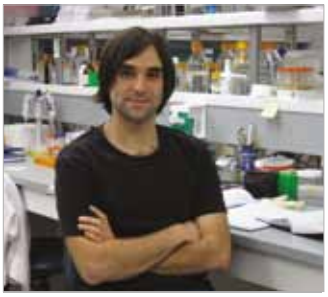
Results:
EPI-002 effectively inhibited constitutive transcriptional activity of AR V7 in PC3 cells as well as combined transcriptional activity of fl-AR and AR V7 in the presence and absence of R1881 in LNCaP cells. EPI-002 in combination with MDV3100 demonstrated greater inhibition of transcriptional activities of AR species than MDV3100 alone. Western blot analysis showed levels of AR protein in cells were unaffected by drug treatments.

Conclusions:
EPI-001 and its stereoisomer EPI-002 are currently the only known compounds that inhibit all AR species including constitutively active AR splice variants lacking LBD. EPI compounds may benefit prostate cancer patients failing current therapies that only target AR LBD.

ALON HENDEL

Supervisor: Dr. David Granville
Session: Basic Sciences

"Granzyme B releases vascular endothelial growth factor from extracellular matrix stores and induces vascular permeability *in vivo*"



AUTHORS:
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Background/objectives:
The formation of unstable and leaky neovessels underlies the pathogenesis of a large number of chronic inflammatory diseases. Granzyme B (GZMB) is a serine protease that is expressed and released by a variety of immune cells and accumulates in the extracellular matrix (ECM) during chronic inflammation where it cleaves a number of ECM proteins, including fibronectin (FN). Vascular endothelial growth factor (VEGF) is a potent vascular permeabilizing agent that is sequestered in the ECM by binding FN in both normal and diseased tissue. We hypothesize that GZMB cleavage of FN will release VEGF from its extracellular stores and promote vascular permeability as a mechanism that contributes to neovessel leakage during chronic inflammation.

Methods:
GZMB-mediated VEGF release from either FN coated wells or endogenously produce endothelial cell (EC) matrix was measured by VEGF ELISA. VEGF-release supernatants were used to treat EC and VEGF receptor 2 (VEGFR2) activation was evaluated by immunoblotting for phosphorylated VEGFR2. Evan's blue was injected intravenously to CD1 mice followed by ear injection of either mouse GZMB, saline control, GZMB + neutralizing mouse VEGF antibody or GZMB+ IgG control (n=5 for each experimental group). Vascular leakage was evaluated by Evan's blue dye extraction.

Results:
GZMB effectively releases VEGF from both FN and from EC matrix, while inhibition of GZMB prevented VEGF release. GZMB-mediated VEGF release resulted in significant activation of VEGFR2 in EC monolayer signified by increased VEGFR2 phosphorylation. GZMB ear injection resulted in a significant increase in vascular permeability *in vivo*. Importantly, co-injection of GZMB and neutralizing mouse VEGF antibody significantly reduced vascular leakage compared to co-injection of GZMB and matching IgG control.

Conclusions:
GZMB increases VEGF bioavailability by releasing it from the ECM leading to VEGFR2 activation and increased vascular permeability *in vivo*. These findings present a novel role for GZMB as a modulator of vascular response during chronic inflammation.

AMAL EL-NAGGAR

Supervisor: Dr. Poul Sorensen
Session: Basic Sciences

"YB-1 contributes to sarcoma metastasis via translational activation of HIF1α"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Sarcomas are a diverse group of malignant neoplasms that are characterized by early metastatic spread, aggressive behavior, and poor prognosis. YB-1 is a member of the highly conserved cold shock domain-containing family of proteins known to bind single and double stranded DNA and single stranded RNA, thereby controlling transcription and translation of a multitude of genes. Our previous studies using a breast cancer model showed that YB-1 promotes an epithelial-to-mesenchymal transition (EMT) in non-invasive breast epithelial cells. YB-1-induced EMT was accompanied by reduced proliferation rates through translational repression of growth-related mRNAs coupled with translational activation of Snail and Twist EMT-related mRNAs. In spite of its role in EMT, comprehensive investigations into the role of YB-1 in the development of sarcomas, which are mesenchymal tumors, are currently lacking. We hypothesize that YB-1, which is known to be elevated in multiple sarcoma subtypes, promotes migration, invasion, and metastasis of sarcoma cells through translational activation of specific transcripts to promote the metastatic phenotype in these lesions.

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 knockdown significantly reduced migration and invasion of each of these cell lines, using in vitro cell motility and invasion assays. YB-1 knockdown also profoundly inhibited migration of GFP-labeled human sarcoma cell lines xenotransplanted into the yolk sacs zebrafish embryos. Then, using an *in vivo* renal subcapsule implantation model previously utilized for epithelial tumors, we found that sarcoma cell lines formed highly invasive local tumors and metastasized to the lungs within 4-6 weeks. However, local invasion and lung spread were almost completely blocked by YB-1 knockdown in the same lines. We then assessed potential mechanisms, and found that Snail and Twist were expressed at low levels in these lines and not prominently regulated by YB-1. In contrast, YB-1 not only directly bound but robustly activated the translation of HIF1α mRNAs, while it had no effects on HIF1α transcription. YB-1 itself was robustly induced by hypoxia, and blocking this induction blocked HIF1α protein levels under hypoxia. HIF1α knockdown blocked YB-1 mediated induction of sarcoma cell migration and invasion, and ectopic expression rescued the effects of YB-1 knockdown on the same parameters. Notably, local implantation site tumors with YB-1 knockdown demonstrated massive hemorrhage and necrosis compared to control tumors, and this was significantly correlated with reduced microvessel density and VEGF production in the former.

Conclusions:
YB-1 promotes sarcoma cell metastasis through translational activation of its downstream mediator, HIF1α, and point to potential consequences for sarcoma angiogenesis. Targeting YB-1 or its downstream effectors represents a promising future target in the treatment of sarcomas.

YU-HSUAN HUANG

Supervisor: Dr. Rusung Tan
Session: Basic Sciences

"SLAM-SAP Signaling promotes the differentiation of IL-17 producing T cells and progression of experimental autoimmune encephalitis"

AUTHORS:
Yu-Hsuan Huang , Kevin Tsai , Rusung Tan And John J. Priatel

AFFILIATIONS:
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Background/objectives:
IL-17 plays critical roles in host defenses, combating bacterial and fungal infections, as well as the pathogenesis of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE). The signaling adaptor SLAM-associated protein (SAP) is essential for normal immune homeostasis as mutations within SH2D1A, the locus encoding this protein, result in serious and sometimes fatal syndromes, including X-linked lymphoproliferative disease (XLP) and severe cases of common variable immunodeficiency. However, the precise cellular bases of how loss of SAP contributes to immune dysfunction remain unclear. To gain further understanding of this genetic disorder, we have utilized a SAP-deficient (Sh2d1a-/-) mouse model system to explore the role of SAP in both the differentiation of IL-17-secreting T cells and IL-17-mediated immune responses.

Methods:
Splenocytes from wild type and Sh2d1a-/- mice were stimulated with anti-TCR and -CD28 or –signaling lymphocyte activation molecule (-SLAM) *in vitro* under IL-17 polarizing conditions and frequency of cytokine producing cells were determined using intracellular flow cytometry. To study the role of SAP in regulating the function of IL-17 producing T cells *in vivo*, we applied EAE model both in wild type and Sh2d1a-/- mice and the pathogenesis and proportions of IL-17 producing T cells were compared.

Results:
CD4 and CD8 T cells lacking SAP had a diminished capacity to differentiate into IL-17-producing T helper (Th17) and T cytotoxic (Tc17) cells relative to wild type. In addition, the use of co-stimulating SLAM antibodies was found to augment the differentiation of IL-17 secreting effectors in wild type but not Sh2d1a-/- splenic T cells under IL-17 polarizing conditions. Furthermore, Sh2d1a-/- mice were protected from EAE and, exhibited greatly decreased numbers of CNS-infiltrating Th17 and Tc17 effector T cells and reduced disease severity.

Conclusions:
SLAM-SAP signaling drives the differentiation, expansion and function of Th17 and Tc17 cells both *in vitro* and *in vivo*.

Poster Presentation | Graduate Student

JAQUES COURTADE

Supervisor: Dr. Bruce Verchere
Session: Basic Sciences

"Alterations in blood glucose homeostasis due to tissue-specific deletion of prohormone convertase 2 (PC2) in islet endocrine cells"

AUTHORS:
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AFFILIATIONS:
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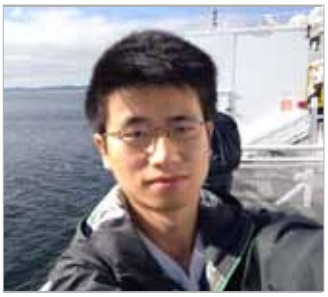
Background/objectives:
Prohormones within the pancreatic islet are processed by a family of prohormone convertase (PC) enzymes essential to the maintenance of blood glucose homeostasis. Prohormone convertase 2 (PC2) is an enzyme essential to the production of insulin, glucagon and islet amyloid polypeptide (IAPP), the primary component of islet amyloid deposits in type 2 diabetes. Along with PC1/3, PC2 is responsible for complete processing of proinsulin and proIAPP and mice with global PC2 deficiency have impaired production of mature insulin and IAPP in beta cells. Despite impaired proinsulin processing, PC2 null mice are hypoglycemic presumably due to the absence of mature glucagon. To better understand the role of PC2 in beta and alpha cells, we rescued PC2 expression in alpha cells of PC2 null mice, and generated mice with exon 4 of the PC2 gene flanked by loxP sites, to enable cell-specific deletion of PC2.

Methods:
PC2 null mice were infected at 8 wk of age by pancreatic duct injection of an adeno-associated virus (AAV) expressing PC2 driven by the rat glucagon promoter (ssAAV6-RGP-PC2). Blood glucose was measured weekly and their pancreas removed for histological analysis at 4 weeks post-infection. Each pancreas was stained with antibodies to PC2, insulin and glucagon. At 8 weeks of age, homozygous PC2-floxed mice were injected with dsAAV8 expressing Cre recombinase driven by the rat insulin promoter (dsAAV8-RIP-Cre) to delete PC2 specifically in beta cells. We measured blood glucose and assessed proIAPP processing by western blot of islet lysates.

Results:
PC2 global knockout mice were hypoglycemic but became normoglycemic (7.4 ± 2.2 vs 11.4 ± 0.8 mM; $p < 0.01$) one week following injection of ssAAV6-RGP-PC2 into the pancreatic duct, suggesting that glucagon production was restored and indicating that alpha cell PC2 deficiency and the resulting inability to produce mature glucagon is the cause of hypoglycemia in global PC2 knockout mice. Immunohistochemical staining revealed colocalization of PC2 with glucagon in the majority of alpha cells and no colocalization of PC2 with insulin, demonstrating the effectiveness of our alpha cell-specific promoter. To assess the role of PC2 specifically in beta cells, we first confirmed successful integration of the PC2 conditional allele into the C57Bl/6 genome by PCR. Homozygous floxed mice injected with dsAAV8-RIP-Cre showed no change in blood glucose compared to wild type controls. By western blot, we observed an increase in IAPP intermediates and decreased mature IAPP, consistent with removal of PC2 in beta cells.

Conclusions:
We have demonstrated that loss of PC2 in alpha cells mediates hypoglycemia in mice with global PC2 deficiency. PC2 activity impacts beta cell prohormone processing but does not affect glycemia, likely due to the ability of PC1/3 to compensate for beta cell loss of PC2 in the processing of proinsulin. These findings suggest that loss of alpha cell PC2 causes hypoglycemia and that while beta cell PC2 plays a role in prohormone processing, it is dispensable for normal glucose homeostasis.



YE QIU

Supervisor: Dr. Decheng Yang
Session: Basic Sciences

"The heat shock protein Hsp70 stabilizes CVB3 genome via interacting with an RNA degradation factor"

AUTHORS:
Ye Qiu[1,2], Xin Ye[1,2], Mary Zhang[1], Paul Hanson[1,2], Maged Hemida[1] and Decheng Yang[1,2]

AFFILIATIONS:
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Background/objectives:
Coxsackievirus B3 (CVB3) is a predominant pathogen of viral myocarditis, an inflammatory disease of the myocardium. The genome of CVB3 is a positive single-stranded RNA, which can be translated directly. The 3' untranslated region (3'UTR) of CVB3 genomic RNA contains an AU-rich element (ARE) that is reported to function as a destabilizing motif for mRNAs. **Heat Shock 70kDa Protein 1A (Hsp70)** is a member of cellular chaperone system that is induced during various cellular stresses. Hsp70 can be translated via a cap-independent mechanism during CVB3 infection, in which translation of mRNAs is usually suppressed by viral proteases. Hsp70 is widely associated with different viral diseases and is involved in cellular energy metabolism, implying a critical role in regulating the heart function. **ARE/poly(U)-binding/degradation factor 1 (AUF1)** is an ARE-binding protein containing four splicing variants (p37, p40, p42, p45), among which p37 and p40 can cause decay of mRNAs containing ARE sites. However, the mRNA decay will be inhibited by interactions between Hsp70 and AUF1. Our study is **aimed** to explore the relationship among Hsp70, AUF1 and CVB3 genome stability. Our **Hypothesis** is that CVB3 infection induces Hsp70 expression that in turn stabilizes viral genome via selective interactions between Hsp70 and different splicing variants of AUF1.

Methods:
HeLa cells were infected with CVB3 and the expression of Hsp70 during viral infection was tested by Western blot. Then Hsp70 was knocked-down by specific siRNAs and different splicing variants of AUF1 were overexpressed in CVB3-infected HeLa cells and HL-1 cardiomyocytes, in order to explore the function of these genes on CVB3 genome. In this step, stability of viral genome was determined by quantifying viral RNAs and viral proteins, using reverse-transcriptional PCR and Western blot, respectively. Finally, to determine that Hsp70 stabilize viral genome via the ARE site, a firefly luciferase reporter was ligated with CVB3 3'UTR containing a wild-type or mutated ARE site and dual-luciferase assay was conducted in Hsp70 knocked-down HeLa cells.

Results:
Protein level of Hsp70 was increased since 3 hours after CVB3 infection. Viral genomic RNA and expression of viral proteins were decreased when Hsp70 was knocked down. Overexpression of AUF1 splicing variants p37, p40 and p42 inhibited expression of viral proteins while overexpression of p45 resulted in increased expression of viral proteins. Knocking-down Hsp70 decreased the expression of a luciferase reporter ligated with a wild-type CVB3 3'UTR while showed no influence on the reporter ligated with CVB3 3'UTR containing a mutated ARE site.

Conclusions:
Our results suggest Hsp70 stabilizes RNA containing CVB3 3'UTR via the ARE site, however AUF1 can either enhance or inhibit CVB3 replication depending on different splicing variants.

Poster Presentation | Graduate Student (Department of Neuroscience)

DHANANJAY NAMJOSHI

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"The liver X receptor agonist GW3965 improves recovery from mild repetitive closed head injury in mice partly through apolipoprotein E"

AUTHORS:
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Background/objectives:
Traumatic brain injury (TBI) increases Alzheimer's disease (AD) risk and leads to the deposition of neurofibrillary tangles and amyloid deposits similar to those found in AD. Agonists of Liver X receptors (LXRs), which regulate the expression of many genes involved in lipid homeostasis and inflammation, improve cognition and reduce neuropathology in AD mice. One pathway by which LXR agonists exert their beneficial effects is through ATP-binding cassette transporter A1 (ABCA1)-mediated lipid transport onto apolipoprotein E (apoE). The goal of this study was to evaluate the ability of LXR agonist GW3965 to promote recovery in a mouse model of mild, repetitive TBI (mrTBI) specifically designed to mimic repeated concussions.

Methods:
We subjected male wild-type (WT) and apoE-/- mice to two mild TBI (mrTBI) spaced at 24 h. Thirty minutes after second TBI, the animals received an intraperitoneal bolus of either GW3965 (20 mg/kg) or vehicle. Mice in the treatment group were thereafter fed with GW3965-containing chow to result an average dose of 15 mg/kg/day for up to 14 days. Mice in the control group were fed with standard rodent chow. Memory and cognition were assessed by novel object recognition (NOR) test. Axonal damage was assessed with silver staining. Endogenous amyloid beta (Abeta) 40 and 42 levels were measured with ELISA. All endpoints were assessed at 2, 7, and 14 days post-mrTBI.

Results:
mrTBI impaired NOR memory of WT and apoE-/- mice in both control and treatment groups within 2 days with no spontaneous recovery by the day 14 day in untreated mice of both genotypes. GW3965 restored memory in WT but not apoE-/- mice by 7 days post-mrTBI. WT mice showed significant axonal damage at 2 day post-mrTBI, which was suppressed by GW3965 treatment. In contrast, apoE-/- mice showed severe axonal damage from 2 to 14 days that was unresponsive to GW3965. Total soluble Abeta-40 and Abeta-42 levels were significantly elevated in WT and apoE-/- within 2 days post-injury. Elevation of Abeta-40 and Abeta-42 was suppressed by GW3965 irrespective of the genotype.

Conclusions:
We found that therapeutic administration of GW3965 improved NOR performance, reduced axonal damage, and suppressed Abeta accumulation after mrTBI. Loss of apoE exacerbated the severity of axonal damage and eliminated the ability of GW3965 to restore NOR performance as well as to promote neuronal recovery. These results are consistent with the role of apoE in neuronal repair and synaptic restoration. Surprisingly, apoE was not required for GW3965 to suppress the transient increase in Abeta levels induced in our model. Our results suggest that both apoE-dependent and apoE-independent pathways contribute to the ability of GW3965 to promote recovery from mrTBI.

Poster Presentation | Graduate Student

ABSTRACT #33

IVY HSU

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

"The extracellular role of granzyme B in wounds of type 2 diabetic db/db mice"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Normal wound healing is a tightly regulated process involving overlapping phases of homeostasis, inflammation, granulation tissue formation and remodelling. However, in diabetics, the normal continuum of these reparative stages is often disrupted leading to the onset of chronic, non-healing wounds. Diabetic wounds are characterized in part by elevated serine protease activity. Granzyme B (GzmB) is a serine protease that can mediate the cleavage and degradation of many extracellular matrix proteins that are important for normal wound healing. We hypothesized GzmB contributes to the pathogenesis of chronic diabetic wounds through the cleavage of extracellular proteins, including fibronectin, vitronectin and decorin.

Methods:
On the lower backs of type 2 diabetic db/db mice, excisional wounds were created. Immediately after wounding and every 2 days for 3 weeks, saline was applied topically on the wounds. Wound closure was monitored and quantified by planimetry to assess epithelialization and contraction. Following euthanasia, skin sections were collected to assess skin morphology and extracellular matrix alterations by immunohistochemistry. GzmB-mediated cleavage fragments were also assessed by western blot.

Results:
Histological analysis of unwounded skin biopsy of db/db mice revealed thinner dermis and thicker hypodermis. Our preliminary studies confirmed delayed wound closure in db/db mice. Increased GzmB expression and co-localization to mast cells, as well as decreased intensity of decorin staining were observed in wounded diabetic skin by immunohistochemistry. Increased fibronectin and vitronectin fragments were detected in wounded diabetic skin by western blot.

Conclusions:
GzmB co-localizes with mast cells in wounded diabetic skin and may promotes cleavage of fibronectin and vitronectin. Future work of this study include inhibiting GzmB by administering Serpina3n, a murine GzmB inhibitor, and assessing skin morphology and ECM alterations by histology and western blot. Results from the study will provide valuable insights into the pathogenesis of and may identify novel therapeutic approaches for the management of chronic diabetic wounds.

Poster Presentation | Graduate Student

JASON HUNG

Supervisor: Dr. Rusung Tan
Session: Basic Sciences

"Novel liposome therapy prevents type I diabetes in non-obese diabetic mice"

AUTHORS:
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Background/objectives:
Type I diabetes (T1D) is an autoimmune disease that affects roughly 300,000 Canadians with the economic burden to Canada reaching $1.2 billion; both are steadily rising. There is no cure and current therapies only treat the symptoms. T1D is caused by a selective breakdown of immunological tolerance towards the insulin-secreting beta cells in the pancreas, leading to uncontrolled regulation of blood glucose levels, which is fatal if left untreated. It is believed that T1D is mainly caused by a dysregulation of immune cells such as natural killer T (NKT) cell, regulatory T cells (Treg) and dendritic cells (DC), which are normally involved in maintaining tolerance to self-antigens and modulating immune responses. In order to restore regulatory function to these cells we have developed a novel liposome that incorporates NKT agonist alpha-galctosylceramide (aGalCer) and insulin, which is thought to be a key antigen involved in T1D and potent activator of Tregs. **We hypothesize that simultaneous activation of DC, Treg and NKT cells through antigen presenting cells using this novel agent prevents T1D in NOD mice**.

Methods:
4 week old female non-obese diabetic (NOD) mice will be injected with the liposomal therapy via 3 different routes of injection: intravenous (IV), intraperitoneal (IP) and subcutaneous (SQ), twice weekly for 6 weeks. To measure the incidence of T1D, blood will be drawn for glucose measurements. The presence of insulin auto-antibodies and antigen-specific T cells linked to T1D (islet specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) and insulin B chain residue 10-18) will also be assessed. Furthermore, cohorts of mice will be sacrificed at various time points and spleen, pancreatic lymph nodes, pancreas and blood will be harvested for frequency and phenotype analysis of NKT, Treg and dendritic cells with flow cytometry. Histology sections of the pancreas will be used to determine insulitis and islet damage.

Results:
We show that the liposomal therapy prevents T1D in NOD mice compared to untreated mice ($p=0.044$) but that route of injection was paramount to the efficacy of the therapy; IV injections provided no protection, IP injections only delayed T1D, while SQ injections fully protected mice from T1D. However, all three routes of injections showed significantly lower levels of IGRP-specific T cells during early time points. Furthermore, all three routes show significantly higher levels of DCs in the spleen, a key organ in immune regulation.

Conclusions:
Dependent on the route of injection, liposomal therapy containing aGalCer and insulin can prevent the development of T1D in NOD mice. We believe that the liposome as well as the route of injection alters the pharmokinetics and pharmodynamics of the therapy, possibly by either increasing the efficacy time, stability or by creating drug reservoirs in different organs. Further studies are currently being done to determine the exact mechanisms, distribution and cytotoxicity of the liposomal therapy. We believe that this type of combinational liposomal therapy offers a new direction of T1D and other autoimmune disease treatment in the future.

JUNYAN SHI

Supervisor: Dr. Honglin Luo
Session: Basic Sciences

"Cleavage of p62/Sequestosome 1 by an enteroviral protease results in disrupted selective autophagy and impaired NFκB signaling"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Autophagy was previously regarded to be non-selective. However, increasing evidence has now suggeted the presence of selectivity in the autophagic degradation of unwanted organelles and aggregate-prone proteins. The adapter protein, p62/sequestosome 1, plays an essential role in mediating selective autophagy. It serves as an autophagy receptor targeting ubiquitinated proteins to autophagosomes for degradation. In addition, it functions as a scaffold protein to regulate signaling pathways via interaction with various proteins. We previously demonstrated that the host autophagy machinery is exploited by coxsackievirus B3 (CVB3) to achieve successful replication. In this study, we aimed to further understand the interplay between CVB3 and the selective autophagy pathway.
Hypothesis: The reduction of p62 protein level following CVB3 infection is due to the cleavage of p62. Cleavage of this adaptor protein will result in disruption of selective autophagy and disregulated signaling pathway.

Methods:
RT-PCR and Western blot were performed to measure the mRNA and protein levels of p62 after CVB3 infection. A series of deletion mutants and point mutants were constructed to identify the cleavage site of p62. In vitro cleavage assay was carried out to test whether viral protease is responsible for p62 cleavage. Confocal and fluorescence microscopy were used to observe the cellular distribution of wide type and cleavage fragments of p62. Dual luciferase assay was used to measure NFκB activity.

Results:
The mRNA level of p62 remained unchanged while protein level was reduced following CVB3 infection. Further investigation found that p62 was cleaved at glycine 241 following CVB3 infection, generating two cleavage products. In vitro cleavage assay demonstrated that the cleavage was executed through the proteolytic activity of viral protease 2Apro. We further showed that the resulting cleavage fragments of p62 were no longer the substrates of autophagy and their ability to form protein aggregates was greatly decreased. Although the C-terminal truncate sustained the binding activity of p62 to LC3, it failed to interact with ubiquitinated proteins. It was also found that co-localization between p62-C and LC3 and ubiquitin within the punctate structures was markedly disrupted. Moreover, we observed that p62-C truncate retained the ability of p62 to stabilize antioxidant transcription factor Nrf2; however, both p62-N and p62-C lost the function of p62 in activating the NFκB pathway.

Conclusions:
Collectively, our results suggest a novel model by which cleavage of p62 as a result of CVB3 infection impairs the function of p62 in selective autophagy and host defense signaling, thereby contributing to viral pathogenesis.

Poster Presentation | Graduate Student (Department of Bioinformatics)

SHAUN JACKMAN

Supervisor: Dr. William Hsiao
Session: Basic Sciences

"Outbreak investigation of salmonella enteritidis using genomic epidemiology"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Outbreaks of salmonellosis regularly occur in BC, and Salmonella enterica serovar Enteritidis (S. Enteritidis) is the most commonly isolated serotype. S. Enteritidis is highly clonal with minor variations across lineages. Subtyping methods currently in use, such as Pulsed Field Gel Electrophoresis (PFGE), therefore have limited discriminatory power for certain S. Enteritidis lineages, and the results are not useful for epidemiological investigations. Whole Genome Sequencing (WGS) can provide detailed information on genomic alterations needed for surveillance and source tracking initiatives. We assessed the strain diversity of outbreak isolates of S. Enteritidis in BC from 1998 to 2011 via WGS.

Methods:
WGS was performed on an Illumina MiSeq platform. All isolates were sequenced to a minimum of 30x genome coverage, sufficient for high quality mapping analysis. 39 isolates of S. Enteritidis, from 10 different outbreaks, for which we have rich laboratory and epidemiological data, were sequenced to gain an initial understanding of strain diversity within and between outbreak strains. Reads were mapped to the S. Enteritidis reference genome P125109 using Burrows-Wheeler Aligner (BWA). Single nucleotide variants (SNV) were called using samtools mpileup. The resulting Variant Call Format (VCF) file was converted to a FASTA file composed of only the genomic positions where isolates differed in genotype. Finally, FastTree was used to to derive a dendrogram from the single nucleotide variants.

Results:
Preliminary SNV analysis demonstrated that we could, in most cases, separate isolates with the same PFGE patterns or phagetypes into clusters corresponding to distinct outbreaks corroborated by epidemiological investigations. SNV analysis and whole genome annotation also provided the resolution necessary to suggest that there might be multiple sources of contamination for some outbreaks, an observation not achievable by traditional subtyping methods or epidemiological investigations alone.

Conclusions:
WGS and SNV analysis are useful tools for assessing and understanding sequence diversity of a highly clonal organism, such as S. Enteritidis. The SNV results can help to guide outbreak investigations if conducted in real-time. By combining genomic data, laboratory surveillance data, and epidemiological data, more detailed narratives can emerge from an outbreak investigation. Further improvements in time and cost of WGS analysis will enable this approach to be performed routinely in public health laboratories.

KYLE BURROWS

Supervisor: Dr. Colby Zaph
Session: Basic Sciences

"The transcriptional repressor HIC1 in CD4$^+$ T cell differentiation and inflammatory bowel disease"

AUTHORS:
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AFFILIATIONS:
[1]The Biomedical Research Centre, 2Department of Cellular and Physiological Sciences and 3Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC

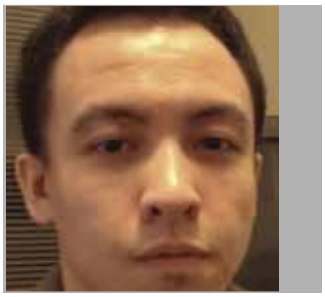
Background/objectives:
Naïve CD4$^+$ T helper (TH) cells can differentiate into several distinct subsets that have specific functions in vivo. TH cell lineage choice is a complex process that requires a balance between transcriptional activation of lineage specific cytokines and transcription factors and silencing of lineage promiscuous genes. Dysregulated TH cell differentiation can lead to the development of many inflammatory diseases such as inflammatory bowel disease(IBD). We hypothesized that the transcriptional repressor Hypermethylated in cancer 1 (Hic1) would have a central role in TH cell differentiation and function.

Methods:
Naïve CD4$^+$ T cells were isolated from spleens and lymph nodes of wild type mice and mice with a specific deletion of Hic1 in T cells (Hic1$^{\Delta T}$ mice) and polarized into different TH cell subsets. Flow cytometry and qPCR were used to assay expression of TH effector cytokines and transcription factors. We use chromatin immunoprecipitation to determine changes in epigenetic marks of activation or repression (histone methylation or acetylation) at specific TH cell loci. In addition, we use the in vivo model of T cell dependent colitis (T cell transfer colitis) to test for physiological relevance in IBD.

Results:
Hic1-deficient CD4$^+$ T cells exhibit heightened TH17 differentiation, with increased expression of Il17A and fail to efficiently differentiate into Foxp3$^+$ regulatory T (Treg) cells in vitro. Although Il17A expression was increased, other T_H17 associated genes such as Rorc and Stat3 showed no significant difference. Epigenetic modifications at the Il17A locus in Hic1$^{\Delta T}$ T cells revealed lower histone 3 lysine 9 (H3K9) di-methylation (repression) and similar H3 acetylation (activation), indicating a more transcriptionally active locus. Following induction of T cell transfer colitis, RAG mice receiving Hic1$^{\Delta T}$ T cells lost significantly less weight than control mice and exhibited decreased pathological symptoms. Analysis of intestinal tissue of the diseased mice revealed significantly lower expression of both Il17A and the Treg-associated gene Foxp3. In addition, there is decreased expression of the pro-inflammatory cytokines Tnfa and Il6. Also, digestion and flow cytommetry of the tissue from the large intestine revealed no CD4$^+$ cells in the gut of Hic1$^{\Delta T}$ recipient mice.

Conclusions:
We have identified a role for Hic1 in the development of T_H17 and T_{reg} cell lineages. As well as identify Hic1 as a central regulator in the development of intestinal inflammation and provide a potential drug target for the treatment of IBD.

Poster Presentation | Graduate Student



ALISTAIR CHENERY

Supervisor: Dr. Colby Zaph
Session: Basic Sciences

"Intestinal worm and microbial interactions during allergic asthma"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
The incidence of allergic diseases such as asthma is becoming increasingly prevalent throughout the world. Canada has one of the highest burdens of asthma with over 2.5 million people affected. The precise environmental and genetic factors that contribute to the etiology of this disease have not been adequately elucidated. However, our understanding of the link between the intestinal immune system and allergic diseases is continually improving. Our aim is to construct an immunological model of the interaction between parasitic infections and the gut microbiota during allergic lung disease. We hypothesized that gut parasites and the host microbiota have an integrative effect on the development and outcome of allergic asthma.

Methods:
A murine dust mite model of allergic airway inflammation was used in combination with acute and chronic intestinal helminth (*Trichuris muris)* infections. Airway disease was assessed by bronchoalveolar lavage flow cytometry, lung histology, and by measurement of inflammatory markers by qRT-PCR and ELISA. Antibiotic treatments were used to characterize the role of the host microbiota during the immune response against *T. muris.* Worm burdens were microscopically counted, inflammatory markers in the colon were measured by qRT-PCR, and cecums were assessed histologically.

Results:
Chronic *T. muris* infection, but not acute infection, caused an exacerbation of airway inflammation based on histopathology (increased inflammatory cell infiltration and epithelial damage). There was also a skewing toward a Th1 immune response both systemically and locally in the airways during chronic *T. muris* infection. This Th1 response was antigen-specific and included an increased interferon-gamma expression and mucus production in the airway. Selective depletion of the microbiota with the antibiotic metronidazole rendered normally resistant C57BL/6 mice to become susceptible to *T. muris*, based on increased worm burdens. Metronidazole-treated mice demonstrated a non-productive Th1 shift in the immune response against *T. muris* and a disruption of goblet cell number and function such as mucus production.

Conclusions:
Our results show that *T. muris*, a helminth parasite that is strictly localized to the colon, can exert systemic effects on the host immune system. The outcome of allergic airway inflammation can be differentially altered based on whether T. muris infection is chronic or acute. We also found a relationship between the host microbiota and the immune response to *T. muris*. Future studies aim to integrate the role of the host anti-parasite immune response with changes in the microbiota and correlate this with the development of allergic lung disease.

SARA SABERI

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Telomere length measurement by a monochrome multiplex qPCR method: assay comparison and optimization"

AUTHORS:
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AFFILIATIONS:
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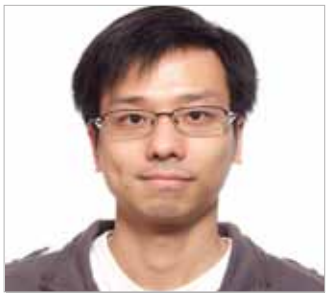
Background/objectives:
Telomeres contain long (GGTTTA)n repeat sequences and protect the ends of eukaryotic chromosomes. As telomeres are not completely replicated, they shorten with each cell division. This eventually leads to cell senescence or apoptosis. Telomere length (TL) thus represents a marker of cellular aging and is frequently measured in human studies. Short leukocyte TL has been associated with cardiovascular disease and shorter lifespan. Current TL measurement methods include Southern blot for measuring Telomere Restriction Fragment (TRF) and flow- fluorescence in situ hybridization (flow-FISH). Both are well established but are labour-intensive and require either large quantities of DNA (TRF) or fresh samples (flow-FISH). The other widely used method is a qPCR-based assay, which exploits a partial mismatch primer strategy. In this monoplex assay two qPCR reactions take place. The telomere SYBR green fluorescence signal (T) in total DNA is quantified and normalized to the copy number of a single copy gene (S) to generate a T/S ratio that is proportional to the average TL. Multiplexing of this assay in a single qPCR reaction is desirable because it eliminates pipetting variability, reduces reagent costs and saves time. The multiplex assay exploits large differences in the relative copy numbers of two DNA sequences (T and S) and in their respective melting temperatures. The signal for more abundant target (T) is captured based on early cycles, when the signal for the less abundant target sequence (S) is still below detection. Considering the small amount of DNA required, the reproducibility and high throughput of this assay, we established and optimized the multiplex assay in our lab for future large-scale human cohort studies.

Methods:
Whole blood (WB) was collected from 32 subjects aged 2 to 59 years. WB DNA was extracted using QIAamp and assayed in duplicate with both the monoplex and multiplex assays on a LightCycler 480. In monoplex, absolute quantification for both T and S was obtained using the LightCycler 480 software v. 1.5. For the multiplex assay, raw text files were converted to grid format using LC480Conversion software and the converted data were analyzed using LinRegPCR software. For both assays, the average leukocyte TL value was calculated as the average of duplicate T*1000 over the average of duplicate of S (T/S). The correlation and reproducibility of the T and S measurements, as well as the T/S ratios of two independent runs by each assay were examined.

Results:
A strong correlation was observed for each individual target (T and S) measured by the two assays (S, $R2=0.95$, $p<0.0001$; T, $R2=0.94$, $p<0.0001$), although the correlation for the T/S ratios was weaker ($R2=0.65$, $p<0.0001$). T/S ratios determined in two independent monoplex and multiplex runs showed a strong within assay correlation ($R2=0.91$, $p<0.0001$ and $R2=0.88$, $p<0.0001$, respectively). Increased variability was observed at the plate's edges with the multiplex assay, especially in the plate corners, something that was not seen with the monoplex assay and may be related to the higher cycling temperatures used.

Conclusions:
A faster multiplex assay was established in our laboratory that showed comparable reproducibility to the monoplex assay at a lower cost and higher throughput.

Poster Presentation | Graduate Student



WAI HANG CHENG

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Comparison of weight drop and supine impactor models as experimental models of mild repetitive traumatic brain injury"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
In developed countries including Canada, traumatic brain injury (TBI) is the leading cause of death and disability for people under 45 years-old. In the United States alone, TBI has an estimated annual incidence of two million. The causes of TBI are diverse, including sports injury, falls, motor vehicle collisions, violence and warfare. Clinically, TBI manifests itself in a large range of severity from severe that may lead to death or extreme disability, or mild, which includes concussions. Although concussions have traditionally thought to be relatively benign, concussions may have an cumulative effect that increases the long-term risk for neurodegenerative disease. The high incidence and complex nature of TBI highlight the need to develop reliable and clinically relevant experimental TBI models for research. This project aims at comparing two types of experimental models that simulate mild closed-head TBI: a gravity driven weight-drop (WD) model and a compressed air supine impactor (SI) model.
Objectives: To compare the acute and long-term outcomes of WD and SI-induced mild repetitive TBI (mrTBI) in young (4-mo) and middle-aged (10-mo) APP/PS1 mice, using histological, biochemical and behavioral analyses.

Methods:
APP/PS1 mice will be anesthetized before WD or SI-induced mrTBI at similar impact energy levels. In the WD model, the scalp is opened by a longitudinal incision to expose the skull. The mouse lies in a prone position with its head under manual restraint, and a weight is dropped onto the parietal bone. The scalp incision is then closed and the mouse is monitored for recovery. In the SI model, no incision is involved, and the mouse lies in a supine position when a pneumatic piston drives a weight onto the head, which allows for free head movement during impact that is recorded with high speed video. In both models, mice will receive two TBI at 24 hours apart, and mice that have skull fracture will be euthanized. Mice undergoing anesthesia but not TBI will serve as sham control. For 14 days post-injury, the Rotarod test will be conducted to monitor motor functions. At 14 days (acute) or 6 months (long-term) after mrTBI, the Barnes' maze test and the Novel Object Recognition test will be performed to assess spatial and object memory, respectively. The mice will then be sacrificed for histological analysis of neuroinflammation, neuronal damage, disruption of axonal functions and deposition of amyloid plaques. Biochemical analyses will be performed to study metabolism of amyloid precursor protein and level of amyloid-beta peptide.

Results:
Diffuse axonal injury, which is commonly observed in clinical TBI cases, results from rotational acceleration or deceleration of the brain, leading to white matter damage in the brain. The WD model restricts head movement during impact, and result in a crushing injury. On the other hand, the SI model may better resemble the clinical situation, as the head is allowed free movement during impact. We expect that the SI model to show more pronounced acute and long-term neuronal and axonal damage in both young and middle-aged mice. In addition, as TBI is hypothesized to increase amyloid-beta production due to the formation of axonal bulbs, we expect that the SI model will lead to more severe Alzheimer pathologies.
Significance: This study may provide insights on critical biomechanical parameters of experimental TBI models that will improve translatability of preclinical TBI research.

DEANNA ZANET

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Current smoking but not previous smoking is associated with shorter leukocyte telomere length, a marker of aging, in HIV$^+$ and HIV$^-$ adults"

AUTHORS:
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Background/objectives:
Smoking is a potent source of reactive oxygen species and leads to inflammation and leukocyte turnover. Smoking, which is highly prevalent in the Canadian HIV+ population, is a risk factor for many aging-related diseases including cardiovascular disease. As leukocyte telomere length (LTL) is a marker for cellular aging, we investigated the effects of smoking and other relevant factors on LTL in HIV+ and HIV- persons who currently, previously, or never smoked.

Methods:
Demographic and clinical information as well as a blood sample were collected from adults enrolled in a cohort studying the effects of HIV therapy on aging. Blood relative LTL was measured by qPCR. Variables univariately related to LTL ($p<0.15$) were used as candidates in the development of multivariate linear regression models.

Results:
LTL was measured in 194 (50%) current- [median (range) age=40 (20-76) years, 74% female, 56% HIV+]; 84 (21%) previous- [34(20-75) years, 76% female, 65% HIV+]; and 112 (29%) never-smokers [34 (20-58) years, 78% female, 54% HIV+]. Candidate variables for the multivariate model for all participants were: age, pack-years smoking, current and previous (vs. never) smoking, illicit drug use ever, active and cleared Hepatitis C virus (HCV) infection (vs. never), HIV+ status, and income <$15,000/year. Due to missing data, parental ages were not included in this model. Among HIV+ participants, HIV-related parameters of disease severity and immune system strength (ie. current CD4 cell count, lowest CD4 cell count ever, and current HIV plasma viral load) were not univariately related to LTL. In a multivariate model for all subjects, older age (Beta=-0.25/10 years, $p<0.0001$), HIV+ status (Beta=-0.13, $p=0.043$), active HCV (Beta=-0.18, $p=0.042$), and current smoking (Beta=-0.19, $p=0.029$) were associated with shorter LTL. In similar models developed for each smoking group, apart from older age ($p<0.005$ in all groups), HIV+ status was only associated with shorter LTL among never-smokers (Beta=-0.47, $p=0.001$) while active HCV showed some association among current-smokers (Beta=-0.17, $p=0.063$). Among previous smokers, no variables other than age were associated with LTL. Overall, pack-years smoking were not related to LTL. There was evidence of potential interactions as HIV status and low income were univariately associated with shorter LTL only among never-smoker participants.

Conclusions:
Half of the cohort participants currently smoked. These results suggest that current smoking status rather than cumulative exposure (pack-years), past smoking or HIV infection impacts LTL most, stressing the importance of smoking cessation interventions.

Poster Presentation | Graduate Student

YUDA SHIH

Supervisor: Dr. Catherine Pallen
Session: Basic Sciences

"The role of protein tyrosine phosphatase alpha in myelination"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Myelin is a fatty material composed of modified plasma membrane that coats axons in the nervous system. This myelin wrapping is critical for neurological function and protects the axons from degeneration. In the CNS, oligodendrocytes are the myelin forming cells. Oligodendrocytes (OLs) mature from a precursor form, oligodendrocyte precursor cells (OPCs), which passes through a series of developmental stages before reaching its mature myelinating phenotype. Disorders of myelin are associated with neurological deficits. Pediatric leukodystrophies are a group of inheritable diseases of myelin in the brain and spinal cord involving reduced or absent myelin. Children born with these incurable diseases have impaired neurological functions; and a key therapeutic goal is to restore myelin by promoting myelination. In addition, acquired myelin defects such as multiple sclerosis are associated with myelin destruction. Therefore, it is important to understand how myelin formation is orchestrated during development and repair. Our lab has previously shown that OPCs derived from mice lacking the cell surface molecule protein tyrosine phosphatase alpha (PTPa) display defects in OL maturation and myelin formation.

Methods:
OPCs were isolated and purified from cerebral cortices of E14.5-E17.5 mice. For this purpose, cortical cells were dissociated and cultured in neural culture media containing epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF) to form neurospheres. Neurospheres were dissociated and cultured in neural culture media containing bFGF and plasma derived growth factor (PDGF) to form oligospheres, which were subsequently dissociated to give single cell OPC suspensions. Dorsal root ganglion neurons (DRGNs) were isolated and purified from the spinal cord of post-natal day 5 mice. DRGNs were cultured for 9 days in vitro (DIV9) on laminin-coated coverslips in DRGN media. OPC suspensions were seeded on laminin-coated coverslips containing DIV9 DRGNs and grown in vitro for 6 and 9 days in co-culture. Coverslips were processed for immunofluorescent staining at DIV6 and DIV9. Coverslips were fixed in 3% paraformaldehyde, permeabilized with Trixton-X-100, blocked in 10% goat serum, and probed with primary antibodies for neurofilament-heavy chain (an axonal protein), and myelin basic protein (MBP), a component of myelin produced by mature OLs. Coverslips were then stained with Alexa-fluor conjugated secondary antibodies and mounted onto slides with Antifade gold containing DAPI.

Results:
OPCs derived from PTPa knockout mice show reduced MBP/NFH co-localization in comparison to OPCs derived from wild type mice in in vitro co-culture following 6 and 9 days in co-culture.

Conclusions:
These preliminary data suggest that PTPa is involved in myelination. However, it is necessary to quantify MBP/NFH co-staining over the entire area of the axonal bed to determine whether there is a significant difference between myelinating abilities of OPCs derived from wild type and PTPa knock out mice.

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:
Many chemical agents, including ones used in cancer chemotherapy, are known to provoke DNA double-strand breaks (DSB's). Moreover, genome instability and defects in cellular DNA repair are hallmarks of many diseases, including cancer. Therefore, there is an interest in identifying genes responsible for DNA DSB repair. Two main mechanisms exist for DNA DSB repair. Non-homologous-end-joining (NHEJ) involves the direct re-ligation of the two broken ends. Homologous recombination (HR), or homologous repair, relies on homologous sequences. The pDR-GFP (direct repeat – green fluorescent protein) construct (Pierce et al.,1999) is widely used to induce DNA DSB at a unique I-SceI restriction site. Successful repair by HR results in an observable GFP signal measurable by flow cytometry. We sought to integrate the pDR-GFP construct and establish a platform using next-generation sequencing (NGS) to directly sequence the repaired sites and measure the NHEJ and HR events. The sensitivity and reproducibility of the system are then tested through biological replicates and gene silencing with known DSB repair genes.

Methods:
pDR-GFP expressing stable cell lines, DR-HCT116 (colorectal cancer) and DR-U2OS (osteosarcoma) were transfected with siRNA. After 24 hr, DNA DSB was induced through I-SceI plasmid transfection, and genomic DNA was extracted 48 hours later. Repair site-specific 600bp amplicons were generated for each sample conditions. Barcoded NGS libraries were then generated and pooled for Illumina MiSeq sequencing. Flow cytometry analyses were also performed to measure %GFP, to compare the results with the NGS-based method. Obtained sequences were then aligned to the original pDRGFP sequence.

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. For NGS-based method, %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 52.9% in non-targeting control to 20.3% and 24.2%, respectively.

Conclusions:
Collectively, these data suggest that the NGS read alignment quantification-based approach provides a more sensitive method to reproducibly measure DNA DSB events. Changes in the frequency of each DSB event can also be measured, at high confidence, following gene silencing of DNA repair genes. Using the same method, a genome-wide siRNA screen would allow us to identify novel DSB repair genes which may be associated with diseases.

Poster Presentation | Graduate Student

MATT ALLARD

Supervisor: Dr. Jiri Frohlich
Session: Clinical Sciences

"Phenotypic characterization and cardiovascular outcomes of patients with familial hypercholesterolemia"

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Background/objectives:
Familial hypercholesterolemia (FH) is a common autosomal dominant disorder caused by loss-of-function mutations in the low-density lipoprotein (LDL) receptor (LDL-R) or apolipoprotein B-100 (apo B) gene, or gain-of-function mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9), resulting in very high blood cholesterol levels and premature cardiovascular disease (CVD).

Objectives: To identify and phenotypically characterize FH patients in the Healthy Heart Prevention Clinic to determine characteristics that increases their risk of developing CVD. Additionally, to obtain data from the BC Vital statistics Registry to determine the causes of mortality in a cohort of treated FH patients, to estimate the effects of changes in treatment efficacy on mortality trends over time, and to examine the implications of these findings for patient management.

Methods:
Perform a chart review of the FH patients in the Prevention Clinic and determine which patients in this cohort developed CVD. This will enable us to determine which patients are particularly susceptible to CVD. Also, the information regarding the cause of death and efficacy of treatment of mortality rate in FH patients will be obtained through the BC Vital Statistics Registry.

Results:
Preliminary work on 447 patients revealed that 110 had evidence of CVD. The average age of these patients (Group 1) is 66.6 with 59.1% being male. The 337 FH patients not found to have evidence of CVD (Group 2) have an average age of 57.5 with 38.9% being male. There were some significant differences (p-value <0.05) in CV risk factors between the 2 groups. In the patients of Group 1 80.7% had a family history of CVD, 61.8 % were current or ex-smokers, 36.4% had history of hypertension, and 13.6% had diabetes. In Group 2 67.4% had a family history of CVD, 35.9% were current or ex-smokers, 17.2% had a history of hypertension, and 3.3% had diabetes.

Conclusions:
There were a number of significant differences between group 1 and group 2. Age, and the frequency of positive family history, hypertension, diabetes, current/ ex-smokers were much higher in patients who had developed CVD. Identifying these risk factors will allow for more aggressive management that can reduce the incidence of CVD.

SAMANTHA HANSFORD

Supervisor: Dr. David Huntsman
Session: Basic Sciences

"Hereditary diffuse gastric cancer: beyond cdh1 mutations"

AUTHORS:
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AFFILIATIONS:
[1]University of British Columbia; [2]Memorial Sloan Kettering; [3]Mount Sinai Hospital; [4]McGill University

Background/objectives:
Gastric cancer is a lethal disease with specific death rates greater than 50%. Although most cases are likely caused by a combination of environmental risk factors and gene environment interactions (sporadic), a small number of cases (<5%) occur through highly penetrant autosomal dominant cancer susceptibility syndromes. Although rare, these syndromes are devastating for affected families and represent powerful cancer prevention opportunities to better understand both inherited and sporadic forms of disease. Since a correlation was made between the cell-adhesion molecule E-cadherin (encoded by the gene CDH1) and hereditary diffuse gastric cancer (HDGC), highly penetrant mutations within the gene have attributed to over 40% of cases (Kaurah et al. 2007). Our team has shown that risk of HDGC can be eliminated through preventative surgery in families where highly penetrant CDH1 mutations have been found. However, the molecular basis of the remaining 60% of families is not known. The objective of this study is to **(1)** use next-generation-sequencing technologies to identify mutations that increase susceptibility to familial gastric cancers and **(2)** obtain somatic evidence in support of the pathogenicity of these candidate mutations.

Methods:
(1) Twenty non-CDH1 HDGC families were screened against a custom designed panel of 55 known upper gastrointestinal (UGI) cancer susceptibility genes (selected based on a literature search) using TruSeq Custom Multiplexed Amplicon assay on a MiSeq sequencer. After prioritizing, candidate variants with the highest expected functional impact (i.e. protein truncating mutations) were validated via Sanger sequencing and segregated within relatives. Families in which no candidate mutation is identified are being sent for whole genome sequencing. **(2)** Tumor samples from mutation carriers will be examined for somatic loss of heterozygosity (LOH) or expression in the candidate familial cancer genes through Sanger sequencing and immunohistochemistry.

Results:
Upon preliminary analysis of 20 non-CDH1 families, novel truncating mutations with high predicted pathogenicity have been identified and validated in 3 cases within the genes CTNNA1, ATM and BRCA2. These genes have each been reported to increase susceptibility of familial UGI cancers through germline mutations and LOH. Segregation within each family is presently underway and tumour samples are currently being located for LOH and functional analysis to further solidify mutation pathogenicity. We have collected an additional 40 CDH1 negative families with strong history of HDGC to undergo this targeted sequencing procedure in hopes of uncovering their genetic susceptibility.

Conclusions:
Based on initial results, some familial gastric cancer cases without CDH1 mutations harbour mutations within other UGI-related genes. The custom designed panel and the TruSeq Amplicon Sequencing assay is an efficient and cost-effective strategy for identifying causative mutations in families with unknown genetic susceptibility. This study will directly and immediately impact the families where new mutations are discovered and will enable predictive testing to be offered to at-risk families, thus decreasing cancer risk.

Poster Presentation | Graduate Student

CHANSONETTE BADDUKE

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

"Contribution of exome sequencing to understanding the 1q21.1 CNV"

AUTHORS:
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AFFILIATIONS:
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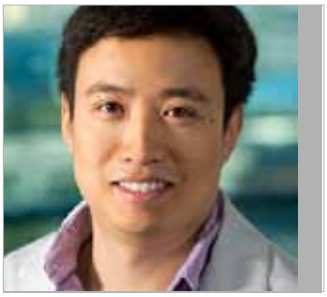
Copy number variations (CNVs) of 1q21.1 are associated with variable physical abnormalities and levels of learning difficulties. We used exome sequencing to look for variants in the 1q21.1 region and genome wide to explain the phenotypic variability seen in two families with 1q21.1 CNVs (3 deletions, and 2 duplications, and 3 unaffected individuals). Sequence variants for follow-up were selected based on minor allele frequency (<1%) in common variant databases (NHLBI 6500 and 1000 exomes), high conservation and pathogenicity scores, and presence in genes with abnormal expression in patient lymphoblasts derived from previous whole genome expression analysis (Harvard et al., Orphanet Journal of Rare Diseases, 6:54, 2010). One InDel leading to a premature stop codon in an endoplasmic reticulum (ER) stress response gene in a father with ADHD and his affected son with developmental delay, both carrying the 1q21.1 duplication, fulfilled all criteria. We used Sanger sequencing to confirm the presence of the variant and qPCR to show that both individuals with the variant have reduced gene expression. We then screened our whole genome expression data for abnormal expression in additional ER stress response genes. Surprisingly, a number of genes from this pathway have reduced expression not only in the two 1q21.1 duplication subjects but also in carriers of the 1q21.1 deletion from the second family, who did not have a detectable pathogenic sequence variation.
Combined whole exome sequencing and whole genome expression findings implicate the ER stress response pathway in carriers of 1q21.1 CNV through pathogenic variants in the stress response pathway and/or perturbations in the function in one of the genes from 1q21.1 CNV affecting the ER stress response pathway. This could lead to phenotypic variability dependent on the level of stress each carrier was exposed to.

JIAMIN CHEN

Supervisor: Dr. David Huntsman
Session: Basic Sciences

"Recurrent DICER1 hotspot mutation in endometrial cancer"



AUTHORS:
Jiamin Chen[1,3], Melissa McMonechy[1,3], Michael Anglesio[1,3], Yemin Wang[1,3], Jiarui Ding[2], Sohrab Shah[2], Andy Chu[4], Gregg Morin[2] and David Huntsman[1,2,3]

AFFILIATIONS:
[1]The BC Cancer Agency; [2]The BC Cancer Research Center; [3]Department of Pathology and Laboratory Medicine, University of British Columbia; [4]The Michael Smith Genome Sciences Centre, Vancouver BC

Background/objectives:
MicroRNAs (miRNAs) are ~ 22 nt single stranded non-coding RNAs that post-transcriptionally regulate gene expression. Alternation of genes associated with miRNA biogenesis pathway may lead to miRNA dysregulation, and is implicated in a variety of human malignancies. Last year, our group identified recurrent somatic "hotspot" missense mutations in a critical miRNA-processing gene, DICER1, in rare sex cord-stromal tumors. During miRNA biogenesis, the two RNase III domains of DICER1 form an intramolecular dimer, which leads to the cleavage of the precursor miRNA (pre-miRNA) hairpin and generate mature 5p and 3p miRNAs from 5' and 3' arms of the precursor hairpin respectively. One strand will be preferentially incorporated into a RNA-induced silencing complex, subsequently promoting the degradation of target mRNAs. In vitro and in vivo studies indicated that the hotspot mutations (E1705, D1709, D1810, E1813) in the RNase IIIb metal binding domain impair DICER1's ability to generate mature 5p miRNAs, leading to global loss of 5p miRNAs. We started to investigate whether the same oncogenic processes were present in other common malignancies. More recently, in collaboration with The Cancer Genome Atlas (TCGA), we identified DICER1 Hotspot mutations in a small subset of endometrial cancer (EC). Two objectives: (i) Establish DICER1 mutational profile and identify miRNA/mRNA signatures associated with hotspot mutations in EC tumors. (ii) Examine the effect of DICER1 hotspot mutations in EC cell line models.

Methods:
Targeted exon sequencing is used to assess DICER1 mutations in EC samples from the Ovarian Cancer Research Program (OvCaRe) tumor bank. Small RNA deep sequencing using the Illumina Hiseq/Miseq systems are applied to profile miRNA expression. Standard Affymetrix Gene expression arrays will be used to profile mRNA expression. Two strategies are used to generate DICER1 null EC cells expressing allelic series of DICER1 hotspot mutations: (i) knock out DICER1 in HEC1A endometrial cancer cells using recombinant adeno-associated viruses; (ii) knockdown of DICER1 using shRNA targeting 3' UTR of DICER1 mRNA. Null EC cells are then transduced using lentiviral vectors expressing DICER1 (hotspot mutations or wild-type controls).

Results:
So far we identified 41 DICER1 mutations including 8 hotspot mutations in 26 cases (6.3%) after sequencing 412 EC samples from provided by our collaborators and OvCaRe. Our pilot miRNA sequencing study suggested that certain 5p miRNAs were indeed reduced in cases with hotspot mutations. Bioinformatic analysis of RNA sequencing profiles from TCGA dataset predicted hotspot DICER1 mutations to have greater functional impact than non-hotspot DICER1 mutations on gene expression. Isogenic EC cell expressing wildtype or mutant DICER1 have been established, and the functional impacts of hotspot mutations are currently under investigation.

Conclusions:
The discovery of DICER1 hotspot mutations in EC implicates DICER1 mutations in a common cancer for the first time. In North America, an estimated 52430 new cases and 8910 deaths due to EC are expected in 2012. Although the hotspot mutations are found in a low frequency, they may represent a unique pathogenic pathway and affect a substantial number of patients.

Poster Presentation | Post Doctoral Fellow

SYAM PRAKASH SOMASEKHARAN

Supervisor: Dr. Poul HB Sorensen
Session: Basic Sciences

"YB-1 translationally regulates stress granule assembly and protects cells from oxidative stress"

AUTHORS:
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AFFILIATIONS:
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The translation and transcription regulator YB-1 is a promising target for cancer therapy. Elevated expression of YB-1 is correlated with poor patient survival rates and drug resistance in various tumor types, and YB-1 levels are significantly elevated in advanced metastatic tumours. YB-1 is localized mainly in three cellular compartments including the cytosol, the mitochondria, and the nucleus. The majority of cellular YB-1 is cytosolic where it binds mRNAs and plays an important role in regulating mRNA translation. Stress granules (SGs) are cytoplasmic inclusions that modulate eukaryotic protein translation. SGs are formed in response to physiological stresses such as oxidative damage, heat shock, hypoxia and starvation, and artificially induced by treatment with H2O2, arsenite, and puromycin. SG formation is a key adaptive mechanism under adverse environmental conditions. Translation is predominantly inhibited under stress conditions and mRNAs stalled in the process of translation initiation assemble as SGs in the cytosol. While YB-1 has previously been associated with SGs, its role in SG biology is unclear. We found that YB-1 indeed localizes to SGs under multiple stress conditions. Moreover, siRNA-mediated knockdown of YB-1 significantly reduces SG assembly in U2OS osteosarcoma and Rh-30 rhabdomyosarcoma cells, in response to two oxidative stress inducing agents, arsenite and H2O2. We found that the association of YB-1 with SGs is largely dependent on its ability to form large oligomers along with other SG proteins TIA-1, G3BP1 and eIF3 in the presence of mRNA. Removal of RNA in live cells inhibited the ability of YB-1 to translocate into SGs. In addition, we found that YB-1 stimulates synthesis of several reported SG proteins that are important for SG nucleation and maintenance. We were also able to show that YB-1 directly binds to and translationally upregulates mRNA encoding G3BP1, a critical SG protein that is necessary and essential for the formation of SGs. Finally, YB-1 knockdown cells with impaired SG assembly are dramatically more sensitive to oxidative stress induced by arsenite or H2O2 treatment.

CANDACE CARTER

Supervisor: Dr. Peter Watson
Session: Clinical Sciences

"Development of a Biobank Resource Centre by the Office of Biobank Education and Research and the Canadian Tumour Repository Network "

AUTHORS:
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AFFILIATIONS:
[1]Office of Biobank Education and Research, University of British Columbia, BC; [2]Manitoba Tumour Bank, University of Manitoba and CancerCare Manitoba, MB, BC; [3]Tumour Tissue Repository, BC Cancer Agency, BC

The Office of Biobank Education and Research (OBER) is a provincial initiative of the Department of Pathology and Laboratory Medicine, University of British Columbia. OBER's goals are: 1) to provide access to education and promote certification of biobanks in British Columbia, throughout Canada and internationally in order to enhance quality through standardization and foster public confidence in biobanks; 2) to facilitate adoption of best practice-based standards through education; and 3) to provide active support for new and established biobanks. To address our third goal, OBER created a Biobank Resource Centre (BRC) in collaboration with the Canadian Tumour Repository Network.

The BRC offers biobank certification, education, services and tools. Certification is a national initiative designed by a group of leading Canadian biobanks to address minimum standards in biobanking across Canada. Ultimately, the program's objective is to increase public confidence in biobanks, minimize risks to research institutions/hospitals and improve the quality of biospecimen collected for research. The comprehensive online education program is comprised of an introductory overview module (that covers the principles underlying biobank operations) and 8 specialized modules. Services available through the BRC include advice and assistance with preparing ethics/regulatory applications, budget proposals and business plans. The BRC team also provides consultation on biobank design, biospecimen processing, management, storage, retrieval infrastructure, facilities design and management. Tools including biobank user fee calculator, biobank inventory database, patient engagement tool and biospecimen data reporting tool are available.

OBER has been established as a one stop shop to communicate common standards and policies amongst biobanks and between biobanks and the public through education, training and support in the form of the BRC.

Poster Presentation | Staff

MOHAMMAD QADIR

Supervisor: Dr. Poul HB Sorensen
Session: Clinical Sciences

"ChildSeq-RNA: a next-generation sequencing-based diagnostic assay for the identification of known gene fusion transcripts in childhood sarcomas"

AUTHORS:
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Several predominantly pediatric sarcomas including Ewing sarcoma (ES), alveolar rhabdomyosarcoma (ARMS), desmoplastic small-round-cell tumor (DSRCT) and congenital fibrosarcoma (CFS) can be extremely difficult to diagnose accurately based on morphology alone. Diagnosis is benefited by the use of ancillary methods such as reverse transcriptase-PCR (RT-PCR) or fluorescence in situ hybridization (FISH) assays to identify pathognomonic gene fusions arising from chromosomal translocations in these tumors. Two major limiting deficiencies associated with RT-PCR and FISH-based assays are, (1) their inability to identify gene fusions at high resolution at the individual nucleotide level, and (2) an inability to identify more than one gene fusion simultaneously. We have developed a next-generation sequencing (NGS)-based diagnostic assay, designated ChildSeq-RNA, that utilizes the Ion Torrent (IonT) sequencing platform to simultaneously screen for gene fusions associated with ES, ARMS, DSRCT and CFS. We have also developed a bioinformatics algorithm to rapidly analyze the NGS data, termed ChildDecode, which operates on a scalable bioinformatics-computing platform. Total RNA from 5 Ewing sarcoma cell lines as well as 27 clinical samples (18 ES, 4 ARMS, 2 DSRCT, 1 CFS, and 2 fusion-negative control cases) were subjected to RNA sequencing using ChildSeq-RNA. This robustly identified EWS-FLI1 or EWS-ERG, PAX3-FOXO1 or PAX7-FOXO1, EWSR1-WT1 and ETV6-NTRK3 gene fusions only in ES, ARMS, DSRCT or CFS cases, respectively, as expected, but with no examples of false positive fusion detection. Results were in 100% concordant with RT-PCR findings, and in addition the assay identified several instances of expression of multiple genotypes of the same fusion in specific cases. We propose ChildSeq-RNA as a novel high-throughput tool for the diagnosis of pathognomonic gene fusions in childhood sarcomas at the single nucleotide level.

CHRISTINE JOSEPH

Supervisor: Dr. Walter Martz
Session: Clinical Sciences

"Establishing the reference range for intraindividual variation of endogenous gamma-hydroxybutyrate (GHB) in hair for B.C. using mass spectrometry analysis - a summary of the "SCOOP" study"

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Background/objectives:
Gamma-hydroxybutyrate (GHB) is categorized into the sedative-hypnotic class of drugs. Due to its physiological effects (ex: central nervous system depression, amnesia), this drug is of particular significance for drug facilitated sexual assaults (DFSA). For forensic purposes, there is currently a lack of a standardized approach to the detection of a suspected, exogenous GHB exposure in alleged cases. GHB is an endogenous neuromodulator that is found in varying concentrations within different biological samples which further augments the analytical complexity of the drug's measurement. As a result of GHB's short half-life, lack of measurable metabolites and narrow detection window, cut-off points for the detection of GHB in blood and urine have not been well established. Consequently, segmental analysis of GHB in hair was proposed because of its potential for drug retention over time. The purpose of this study was to determine if the population-based variation of endogenous GHB in scalp hair samples from individuals within B.C. (not claiming exposure to GHB) is large enough to falsely indicate an exposure to exogenous GHB in DFSA using liquid-chromatography tandem mass spectrometry (LC/MS/MS) analysis.

Methods:
A total of 106 hair specimens from individuals were collected. These sample profiles were washed in methanol, hot water, followed by dichloromethane. The dried hair was then cut into 10 sequential (3mm, 10 mg) segments and digested with 1N NaOH at 85 degrees Celsius. GHB was isolated from these samples using a liquid-liquid extraction technique with ethyl acetate as the organic solvent. Standards, matrix blank and quality controls were also prepared from blank (10 mg) hair samples and these were subjected to the same extraction procedure. All of the samples were analyzed using the LC/MS/MS 4000 QTrap (ABSciex) instrument and deuterated GHB (GHB-D6) was the internal standard for each run. Mass spectrometry analysis was through atmospheric pressure chemical ionization (APCI) in negative mode with multiple reaction monitoring (MRM). Calibration curves were constructed from standards and the GHB concentration was extrapolated for each hair segment. The limit of quantification (LOQ) for GHB with this method is 0.1 ng/mg of hair.

Results:
Statistical evaluation of the baseline levels of GHB for the 106 profiles of segmented hair demonstrated a mean concentration of 1.35 ng/mg (0.00-10.00 ng/mg). The relative standard deviation (RSD) was highly variable among these individuals with an average RSD of 36.7%.

Conclusions:
The collected data serves as a reference point for residents in B.C. It also demonstrates the use of hair samples as the biological matrix to detect exposure to GHB, particularly in forensic casework where the time frame between drug exposure and sample analysis maybe prolonged. Based on our results, considerable intraindividual variation of endogenous GHB levels can occur, which is consistent with previously published data.

Poster Presentation | Undergraduate Student

DAVID ROWBOTHAM

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

"Elf3, a fetal lung transcription factor, is frequently re-activated in lung adenocarcinoma"

AUTHORS:
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AFFILIATIONS:

Background/objectives:
To identify novel putative oncogenes in lung adenocarcinoma (AC), frequently activated by copy number gain and hypomethylation, that could be potentially exploited as therapeutic targets or clinical markers.

Methods:
ELF3 was identified as frequently gained, hypomethylated and overexpressed in a panel of 83 AC samples as compared to their non-malignant equivalents. Copy number analysis was performed using the SNP 6.0 platform, methylation analysis was performed using the Illumina HM27 Methylation platform, and expression analysis was performed using the Illumina expression microarray. A U-test was performed comparing ELF3 methylation between AC and normal tissues ($p<0.05$). Subcellular localization of ELF3 was determined by western blot on nuclear and cytoplasmic fractions, as well as by immunofluorescence. The role of ELF3 in cell viability and the ability to form colonies in soft agar was assayed using stable ELF3 mRNA knock-down in AC cell lines with high ELF3 expression. Knock-down was verified by qRT-PCR and western blot. Genes likely regulated by ELF3 were identified by genome-wide expression analysis using the Illumina HT-12 BeadChip microarray comparing knock-down cell models to empty vector controls. Correspondingly affected biological functions and pathways were elucidated using Ingenuity Pathway Analysis software.

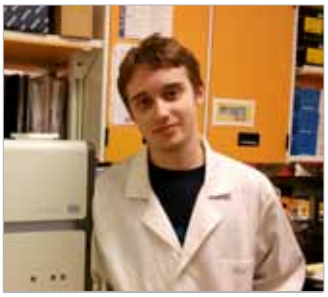
Results:
ELF3 was found to be frequently gained (49%), hypomethylated ($p<0.05$), and overexpressed by at least 2-fold (72%) in AC. Knock down of ELF3 resulted in decreased cell proliferation as well as formation of fewer and smaller colonies in soft agar. Western blots indicated ELF3 is localized to the nucleus, which was confirmed by immunofluorescence. Its activity as a transcription factor was supported by cell model expression data demonstrating deregulated expression of additional transcripts when ELF3 is overexpressed.

Conclusions:
ELF3 is a gene frequently disrupted by genetic and epigenetic mechanisms, which result in its overexpression in AC. ELF3 plays roles in cell proliferation as well as contact-independent growth, likely through its transcription factor activity. Oncogenic transcription factor activity is supported by subcellular localization to the nucleus and expression data. The clinical utility of this protein must be interrogated.

ADAM ZIADA

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Measuring somatic mitochondrial DNA point mutations in relation to aging and HIV infection"



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Background/objectives:
Over 30 million people in the world live with HIV. Despite the great success of antiretroviral therapy at preventing AIDS-related deaths, people living with HIV seem to experience accelerated aging, expressed through earlier onset of age related diseases such as 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. These mutations, including somatic mtDNA point mutations, are implicated in many age-associated diseases such as those seen in HIV-infected individuals. HIV infection itself, as well as antiretroviral agents, can increase oxidative stress, and may contribute to accelerated aging.
Objective: In this pilot study, we propose to investigate whether somatic mtDNA point mutations can be used as a biomarker for biological aging, and whether there is an association between HIV status and somatic mtDNA point mutation burden.

Methods:
As somatic mtDNA point mutations are challenging to measure because of their low frequency, often at or below the background level of most mutation burden assays, 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 GS Junior 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 looked like. True mutations should be present in close to 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 will be studied, along with clonal plasmid DNA (no mutations control) (n=5). Somatic mtDNA point mutations will be 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).

Results:
Preliminary data suggest that blood mtDNA mutation levels measured in neonates and adults with this assay are above background levels. Study samples are currently being analyzed and the results will assist in power calculations.

JEFF ZONG

Supervisor: Dr. Decheng Yang
Session: Basic Sciences

"Role and regulation of heat shock 70 kDa protein 1A (Hsp70) in coxsackievirus B3-induced myocarditis"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
Myocarditis is an inflammatory disease of the myocardium which may progress to dilated cardiomyopathy and heart failure. Multiple etiologies have identified, enterovirus Coxsackie B3 (CVB3) being one of the most common causes of myocarditis. CVB3 has a positive, single-stranded RNA genome encoding a single long open reading frame flanked by a 5' untranslated region (UTR) containing an internal ribosomal entry site and a 3' UTR harboring an A-U rich element (ARE) with a poly-A tail . ARE has been shown to destabilize cellular mRNAs via the recruitment of A-U rich RNA-binding factor-1 (AUF-1). Hsp70 is a heat shock protein and plays essential roles in stress conditions including certain viral infections. It was shown that Hsp70 induction reduces degradation of ARE-mRNAs by sequestering AUF-1 and suppressing its binding to ARE. Therefore, Hsp70 may take a crucial part in CVB3 RNA replication. This project investigates if Hsp70, induced by CVB3 infection via its transcription factor HSF-1, promotes CVB3 replication by stabilizing the viral RNA genome.

Methods:
HeLa cells were infected with CVB3 or sham infected with PBS, and cell lysates were collected at different time points. The changes in Hsp70, HSF-1, and AUF-1 expression levels were monitored by Western blot (WB). To study the effect of HSF-1, Hsp70 and AUF-1 on CVB3 replication, small interfering RNA (siRNAs) were applied to silence these three genes separately in HeLa cells. Cells were then infected with CVB3. Viral replication was evaluated by WB detection of viral proteins and viral plaque assay of CVB3 particle formation and quantitative RT-PCR detection of viral genomic RNAs. To study the CVB3 genome stability, cells treated with Hsp70-siRNA or AUF-1-siRNA and infected with CVB3 will be subjected to Northern blot analysis of CVB3 RNA integrity and luciferase assay of reporter gene translation.

Results:
WB results showed that Hsp70 and HSF-1 were upregulated during CVB3 infection. Three of the four AUF-1 isoforms (p40, p43, p45) were suppressed by CVB3 infection with exception of the p37 isoform, which also showed resistance to siRNA treatment. Knocking down of Hsp70 suppressed CVB3 replication, which is evidenced by lower viral protein levels and less viral progeny release. Knocking down of HSF-1 inhibited the induction of Hsp70, downregulated the viral protein expression and preserved the AUF-1 level during CVB infection, but showed increased viral progeny via plaque assay. Moreover, knocking down of AUF-1 slightly decreased CVB3 protein and viral progeny release during infection.

Conclusions:
CVB3 infection induces Hsp70 upregulation through HSF-1 activation, which is beneficial for viral replication.

JONATHAN MONG

Supervisor: Dr. Miguel Imperial and Dr. Linda Hoang
Session: Clinical Sciences

"Exploring the use of next generation sequencing as a tool in the investigation of a C. *difficile* epidemic"

AUTHORS:
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AFFILIATIONS:
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[2]Department of Pathology and Laboratory Medicine, UBC

Background/objectives:
Clostridium difficile is known to be an important enteric pathogen associated with antibiotic-induced diarrheal disease that has a significant rate of morbidity and mortality. Next-generation sequencing (NGS) offers the possibility of distinguishing isolates at a higher resolution than pulsed-field gel electrophoresis(PFGE); as such, the objective of this study was to explore the possibility of using NGS to track an outbreak of C. diff and compare it to the data obtained through PFGE, the current gold standard.

Methods:
We performed next-generation sequencing using an Illumina MiSeq kit on six isolates from an evolving hospital outbreak of C. difficile associated diarrhoea (CDAD), as well as on five historical isolates from an outbreak that occurred in 2008. The results were analyzed using Galaxy Mapping Software, and a dendrogram based on single nucleotide polymorphism (SNP) differences from the NGS data was constructed and compared to the epidemiologic data as well as the results obtained from PFGE.

Results:
NGS was able to group four of the concurrent outbreak isolates into a distinct cluster, which closely matched the epidemiologic data. The isolates contained within this cluster came from patients who had been in close proximity with each other during the outbreak. The other two samples, which had been obtained from patients who were temporally and spatially separated, were not shown to be genetically related. Similarly, three of the historical isolates formed a cluster.

Conclusions:
The results suggest that the use of NGS as a tool for epidemic investigation is feasible, and offers a higher degree of resolution than the current standard, PFGE, allowing infection control workers to make more informed and precise decisions during and after an outbreak.

Poster Presentation | 2012 Pathology Summer Studentship Recipient

AMANZO HO

Supervisor: Dr. Michael Allard
Session: Basic Sciences

"Cardiac hypertrophy and fibrosis with conditional cardiac-specific deletion of LKB1 and modulation by exercise in adult mice"

AUTHORS:
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Background/objectives:
Fibrosis is a characteristic feature of pathological cardiac hypertrophy, a form of cardiac hypertrophy associated with heart dysfunction and failure. This is opposed to exercise-induced physiological cardiac hypertrophy, which typically shows no increased fibrosis or cardiac dysfunction. Studies have implicated the liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) pathway in the development of both pathological and physiological cardiac hypertrophy. In the pathological form, this pathway is believed to be a negative modulator of hypertrophy. However, the exact role of LKB1 has yet to be fully elucidated. This study aims to quantify the changes in cardiac fibrosis and heart weight following cardiac-specific deletion of LKB1 in adult mice. The modulating effects of exercise on cardiac fibrosis and heart weight were also investigated.

Methods:
Adult (6 weeks old) mice with tamoxifen-inducible cardiac-specific Cre recombinase knock-out of LKB1 (LKB1-cKO) were randomly assigned to receive tamoxifen (0.1 mg/day, i.p.) or vehicle (corn oil) over a 4-day treatment period. Two groups containing LKB1-cKO mice treated with tamoxifen or vehicle were allowed to voluntarily exercise via a running wheel in their cage. Mice were euthanized at 10 weeks post-treatment and hearts were harvested, weighed, and fixed in 10% formalin. Hearts were sectioned and histologically stained with picrosirius red to visualize collagen for quantification of fibrosis using colour segmentation computer software (Image Pro Plus 5.1).

Results:
Non-exercising (Idle) mice with conditional loss of cardiac-specific LKB1 showed significantly increased heart weight (16.9 ± 5.5%, normalized to tibial length) and significantly increased collagen in the right (63.8 ± 11.8%) and left (76.0 ± 9.2%) ventricles compared to vehicle-treated LKB1-cKO mice. Exercise also caused significantly increased heart weight (39.0 ± 4.4%) in vehicle-treated LKB1-cKO mice, but produced no significant change in collagen. Exercise in mice with loss of cardiac-specific LKB1 also resulted in significantly increased heart weight (14.0 ± 3.2%), but the extent of increase was blunted compared to that of vehicle-treated LKB1-cKO mice. Exercise following LKB1 deletion resulted in significantly less collagen in both the left (-62.5 ± 5.5%) and right ventricles (-76.5 ± 10.8%) compared to idle LKB1-cKO mice. There was no significant difference in heart weight or collagen between wild-type (C57 BL6) and LKB-cKO mice treated with vehicle. Tamoxifen caused no significant change in collagen or heart weight in wild-type mice.

Conclusions:
Post-natal deletion of cardiac-specific LKB1 causes cardiac hypertrophy and fibrosis. Alternatively, exercise produces cardiac hypertrophy without increased fibrosis. These results suggest the LKB1/AMPK pathway negatively regulates aspects of pathological cardiac hypertrophy. Exercise mitigates development of cardiac fibrosis accompanying the loss of cardiac-specific LKB1 and its effect on heart mass may be influenced by the LKB1/AMPK signaling pathway.

COLLEEN FOSTER

Supervisor: Dr. Christopher Dunham
Session: Basic Sciences

"Overexpression of PLK1 predicts poor outcome in pediatric medulloblastoma"

AUTHORS:
Colleen Foster[1,2], Abbas Fotovati[2], Cathy Lee[2], Joanna Triscott[2], Katherine O'Halloran[2], Ash Singhal[2], S. Rod Rassekh[2], Sandra Dunn[2], Christopher Dunham[2]

AFFILIATIONS:
[1]UBC Department of Pathology Summer Studentship Program; [2]Children's and Women's Health Centre of B.C.

Background/objectives:
Recent research has suggested the presence of 4 prognostically significant and distinct molecular subgroups of pediatric medulloblastoma: Wnt, SHH, Group 3 and Group 4. Wnt tumors are associated with better outcome while Group 3 tumors exhibit worse prognosis. Surrogate immunohistochemistry (IHC) based approaches to molecular subtyping have been suggested, but appear to lack reliability across labs. NanoString's nCounter Analysis System (nCAS) has been touted as a more reliable and cost effective manner of molecular subtyping. Despite these advances in molecular subtyping, the pathogenesis of medulloblastoma is still unclear. Brain tumor initiating cells (BTIC) have been proposed to play a role in the pathogenesis of medulloblastoma, especially in recurrent cases. Notably, the potential interplay between molecular subtyping and BTICs has yet to be fully investigated. Polo-like kinase 1 (PLK1) is a suspected BTIC gene whose expression, in small cohorts, has been found to be higher in medulloblastoma.

Methods:
We performed a retrospective clinicopathologic analysis of 81 medulloblastomas treated at Children's and Women's Health Centre of BC from 1986-2011. A tissue microarray (TMA) was constructed at the BC Cancer Agency (BCCA). IHC was performed on the TMA using proposed molecular subtype specific antibodies: B-catenin (Wnt), SFRP1 (SHH), NPR3 (Gp 3), KCNA1 (Gp 4). IHC for PLK1 was also performed. Each IHC stain was scored by 2 blinded reviewers into "high" and "low" expressing cases based on staining intensity and diffusivity. Molecular subtyping via nCAS was performed at BCCA using mRNA extracted from FFPE blocks. nCAS was also used to evaluate the mRNA levels of PLK1. nCAS and IHC data were then compared to evaluate the best means of medulloblastoma subtyping. Kaplan Meier survival and multivariate Cox regression analyses were conducted using SPSS (version 20).

Results:
Molecular subtyping via IHC demonstrated that only B-catenin staining had reliable sensitivity and specificity; overall IHC methods failed to accurately subtype cases. In contrast, molecular subtyping by nCAS was very robust, with each resection demonstrating a distinct and non-overlapping subtype. Using the nCAS data, Kaplan Meier survival analysis revealed a significant difference in 5 year overall survival (5 yr. OS) between the 4 molecular subtypes ($P < .001$), with SHH and group 3 cases demonstrating particularly poor outcome. It was possible to assess PLK1 immunohistochemical activity in 67 cases. "High" PLK1 expression (31/67) was significantly associated with worse 5 yr. OS and event free survival ($P < .002$ and .005); similar results were achieved using PLK1 expression data from the nCAS analysis. Interestingly, although PLK1 expression was generally elevated across subtypes, significantly higher expression was particularly associated with SHH subtype ($P < .05$).

Conclusions:
Molecular subtyping of medulloblastoma by nCAS was found to be superior to IHC. In addition, and in keeping with the literature, molecular subtyping carried prognostic significance (Group 3 and SHH tumors are associated with worse overall survival compared to Wnt and Group 4 tumors). With respect to markers of BTICs, medulloblastomas demonstrating particularly "high" PLK1 IHC expression were associated with SHH subtype and poor outcome. These initial analyses support a potential clinical management strategy involving chemotherapeutic manipulation of PLK1 in the SHH subset of pediatric medulloblastomas.

Poster Presentation | 2012 Pathology Summer Studentship Recipient

ALVIS YU

Supervisor: Dr. Patrick Tang
Session: Clinical Sciences

"Epidemiology of non-tuberculous mycobacterial species in BC"

AUTHORS:
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AFFILIATIONS:
[1]University of British Columbia; [2]BC Center for Disease Control

Background/objectives:
All *Mycobacterium* species recovered in BC are handled by the BCCDC Public Health Laboratory and the clinical management is done in consultation with BCCDC TB Control. In order to speciate non-tuberculous mycobacteria (NTM), DNA sequencing of the hsp65 gene is performed on all isolates. However, over the past 7 years, epidemiological changes of NTM in BC have not been characterized. Novel NTM species, which have routinely been recovered from clinical specimens over the same time period have also not been characterized.

Methods:
We gathered all the hsp65 sequence data from 2005-2012 and created a database to store and organize the sequences. We then utilized basic bioinformatics techniques to analyze these DNA sequences including sequence alignment and phylogenetic analysis. Novel species (i.e., those not yet described in NCBI GenBank) were identified and matched with clinical data retrieved from BCCDC TB Control. Novel species found to cause significant infection requiring treatment were sent for whole genome sequencing. The epidemiology of each Mycobacterium species in BC from 2005-2012 was also determined.

Results:
When the novel species identified in 2012 were matched with clinical data retrieved from BCCDC TB Control, one novel species whose hsp65 sequence data most closely matched to Mycobacterium asiaticum was found to cause an infection requiring multiple courses of antibiotics and ultimately, admission into hospital for IV antibiotic therapy. This novel species was sent for whole genome sequencing and is currently being annotated The incidence of NTM infections were determined from 2005 to 2012. Most notably, there have been increases in the incidence of specific Mycobacterium species.

Conclusions:
The epidemiology of non-tuberculous mycobacteria in BC has changed over the past 7 years, including the introduction of clinically relevant novel species. The environmental factors leading to these changes have not yet been explored. Future work will include determining the factors driving NTM rates in BC and further characterization of the novel NTM species.

DIANA LAM

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

"The role of granzyme B in modulating VEGF bioavailability"

AUTHORS:
Diana Lam[1], Alon Hendel[1,2], David J. Granville[1,2]

AFFILIATIONS:
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Background/objectives:
Angiogenesis, the formation of new capillaries from pre-existing vessels, is an important process that supports tissue growth and repair. Dysregulation of angiogenesis plays a role in a number of diseases including cancer, chronic obstructive pulmonary disease, delayed wound healing and rheumatoid arthritis. A major regulator of angiogenesis is vascular endothelial growth factor (VEGF). Low microenvironmental levels of VEGF support physiological angiogenesis. Conversely, high VEGF levels may promote pathological angiogenesis characterized by enlarged, irregular, leaky microvasculature that are commonly observed in chronic inflammatory diseases. VEGF levels are regulated by the extracellular matrix (ECM) as VEGF is sequestered by fibronectin (FN), an important ECM protein that binds VEGF. Matrix-bound VEGF results in regulated angiogenesis, while release of VEGF from the matrix is shown to dysregulate normal angiogenesis. Our lab is focused on granzyme B (GZMB), an immune-derived protease that is observed in a number of chronic inflammatory diseases that are associated with pathological angiogenesis. GZMB cleaves a number of ECM proteins, including FN. We hypothesize that GZMB promotes pathological angiogenesis through modulating VEGF bioavailability by releasing it from the ECM.

Methods:
VEGF release assay was used to determine whether GZMB-mediated FN cleavage leads to VEGF release. Purified plasma FN and cell-derived FN from human microvascular endothelial cells (HMVEC) were tested. Quantification of VEGF release was determined by ELISA.

Results:
GZMB increased VEGF release from purified plasma FN and cell-derived FN compared to control.

Conclusions:
Based on our results, we concluded that GZMB can release VEGF from the ECM by cleaving plasma and cell-derived FN. GZMB may play a role in promoting pathological angiogenesis during chronic inflammation by modulating VEGF bioavailability.

Poster Presentation | 2012 Pathology Summer Studentship Recipient

SZE WING WONG

Supervisor: Dr. Rusung Tan, Dr. Ashish Marwaha
Session: Basic Sciences

"Determining polarization conditions to convert FOXP3+IL-17A+ T cells to Th17s or Tregs"

AUTHORS:
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AFFILIATIONS:
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Background/objectives:
A defective regulatory T cell (Treg) population and an increased pro-inflammatory T helper 17 cell (Th17) population have been associated with a variety of autoimmune diseases, including type 1 diabetes. Recently, human Tregs expressing intermediate levels of their lineage specific transcription factor FOXP3, with measurable suppressive function, have been found to produce IL-17, a cytokine characteristically secreted by Th17 pro-inflammatory cells and to express the Th17 lineage specific transcription factor RORC2. We have previously described an increased proportion of FOXP3+IL-17+ T cells (Th17/reg cells) in the peripheral blood of patients with newly onset type 1 diabetes. Given the phenotypic similarities Th17/reg cells share with both regulatory T cells (Tregs) and Th17 pro-inflammatory cells, we hypothesized that the increased subset of Th17/reg cells isolated from patients with type 1 diabetes can be polarized to either a regulatory (Treg) or inflammatory (Th17) cell lineage.

Methods:
Peripheral blood mononuclear cells (PBMCs) and sorted CD14-CD4+CD45RA-CD25intermediateCD127lowCCR6+CD161+ T cells were placed in human culture media and stimulated with anti-CD3/CD28 dynabeads for several days under the following conditions: (a) the addition of reagents to favour Treg differentiation; (b) the addition of reagents to favour Th17 differentiation; and (c) neutral conditions with no addition of any polarizing reagents. The cultured cells were analyzed by flow cytometry following phorbol 12-myristate13-acetate (PMA)/ionomycin/Brefeldin A stimulation. Tregs were identified as CD4+FOXP3high T cells and Th17s were gated as CD4+IL-17+ T cells.

Results:
In PBMCs, il-2, rapamycin and TGF-beta promoted Treg polarization while 6-formylindolo[3,2-b]carbazole or il-23 supported Th17 polarization. In sorted cells, addition of IL-2 (100U/ml) and rapamycin (100ng/ml) promoted slight Treg polarization, while stimulating with anti-CD3/CD28 dynabeads alone supported Th17 polarization.

Conclusions:
The improper functioning of T cells in type 1 diabetes remains a topic of current research. Treatment of the disease can possibly come from using Treg polarizing reagents to promote proper function or through the blockage of Th17 progression.

Poster Presentation | 2012 Pathology Summer Studentship Recipient

ABSTRACT #61

ELENA CAVAZZI

Supervisor: Dr. Jacqueline Quandt
Session: Basic Sciences

"The antioxidant TEMPOL limits blood-brain barrier alterations in models of multiple sclerosis"

AUTHORS:
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Background/objectives:
Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by demyelination, axonal loss, and ultimately neurodegeneration that leads to disability. Oxidative damage attributable to free radicals is thought to contribute to several steps in the pathogenesis of the disease, including alterations at the level of the microvasculature or blood-brain barrier (BBB) that increase vascular permeability and immune cell recruitment. We have shown that oral administration of the antioxidant TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), a nitroxide radical with anti-inflammatory and cytoprotective properties, limits clinical disease in experimental autoimmune encephalomyelitis (EAE), the animal model of MS. We hypothesize that TEMPOL acts to regulate activation of the cerebrovasculature and immune cell entry during the early stages of inflammation, thereby reducing the severity of clinical symptoms.

Methods:
Using human brain microvessel endothelial cells (HBMEC) as an in vitro model of the BBB, we examined whether TEMPOL could limit the expression of cell adhesion molecules and co-stimulatory molecules in response to inflammatory mediators. Protein was quantified and compared by ELISA, flow cytometry and western blotting.

Results:
Immunohistochemistry and flow cytometry showed significant reductions in immune cell infiltrates, especially macrophages, in TEMPOL-fed EAE animals compared to controls both at peak disease and shortly after the onset of symptoms. TEMPOL alone (50-800 uM) had no effect on constitutively low or negligible expression of E-selectin, or intercellular/vascular cell adhesion molecule (ICAM-1, VCAM-1) expression. Higher doses of TEMPOL limited the upregulation of VCAM-1 in response to tumor necrosis factor alpha (TNF-alpha) but had variable effects on CAM expression in response to lipopolysaccharide. TEMPOL limited upregulation of class II MHC in response to interferon gamma (IFN-gamma) in a dose dependent manner. Further studies are ongoing to compare TEMPOL to other antioxidants in their ability to limit BBB co-stimulatory molecule upregulation in response to inflammatory stimuli and oxidative damage.

Conclusions:
TEMPOL's ability to limit endothelial activation and perhaps also immune cell recruitment, each important precursors to CNS demyelinating and neurodegenerative disease, may contribute significantly to the therapeutic potential of nitroxide radicals in MS.

Poster Presentation | 2012 Pathology Summer Studentship Recipient

SEOK JAE (JOHN) BANG

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Elucidating the mechanisms by which progestins increase astrocyte apoE secretion"

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Alzheimer's disease (AD) is the most common cause of late-onset dementia. Two major risk factors for AD are age and apoE genotype. In the brain, apoE is mainly produced from astrocytes and one of its functions is to facilitate the removal of amyloid beta peptides that accumulate in the AD brain. One mechanism by which apoE is regulated is via a class of nuclear receptor transcription factors called liver X receptors (LXR). Pharmacological approaches to increase apoE levels by selective LXR activation show cognitive benefits in AD mice, but these are not feasible in humans due to significant side effects such as hepatotoxicity. Thus, new ways to modulate apoE levels are desirable. From a high throughput screening effort, the progestins lynestrenol and progesterone were found to increase astrocyte apoE secretions. Because progestins classically act through progesterone receptor (PR) signaling, the goal of this experiment was to determine the mechanism underlying this increase in apoE secretion by blocking PR signaling. This was achieved using the PR antagonist, RU486. Preliminary results suggest that at least some of the increase in apoE secretion was mediated by PR signaling, especially with lynestrenol.

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