a place of mind

PATHOLOGY
laboratory medicine
FACULTY OF MEDICINE
UNIVERSITY OF BRITISH COLUMBIA

PATHOLOGY DAY 2015

Abstract Book Posters & Oral Presentations

May 22, 2015

Featuring oral and poster presentations from graduate students, residents and other trainees

KEYNOTE LECTURE
B. BRETT FINLAY

JAMES HOGG LECTURE
DONALD E. BROOKS

GUEST SPEAKER
CHERYL WELLINGTON

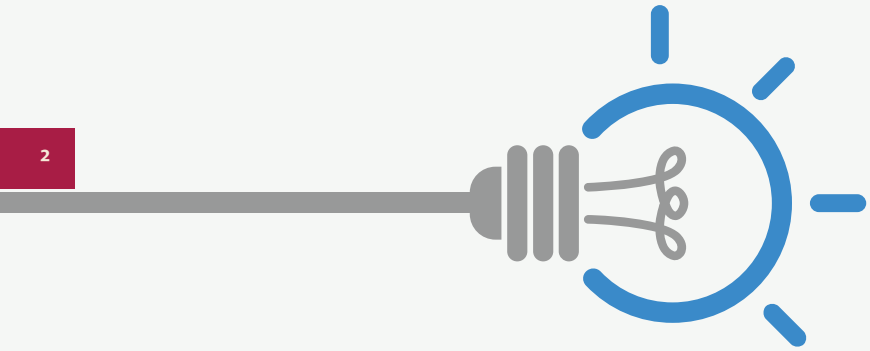
GUEST SPEAKER
DAVID GRANVILLE

GUEST SPEAKER
HUGO HORLINGS

Acknowledgements

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2015 edition.

Gayla Johnson, who is no longer in the Department, Zunaira Saleem and Adeline Chan have been instrumental in handling the administrative and practical details of Pathology Day. Debbie Bertanjoli designed the website and managed the website tools in addition to preparing the abstract book.



PLANNING COMMITTEE

Corree Laule,
Assistant Professor

Avi Ostry, Clinical
Clinical Associate
Professor

We also wish to express our gratitude to the many department members who contributed their time and expertise to reviewing abstracts, moderating the oral sessions, and judging the oral and poster presentations.

This year these include: David Schaeffer, Deborah McFadden, Doug Filipenko, Kevin Bennewith, Diana Ionescu, Jiechuang Su, Jerome Robert, Masud Tehmina, Jane O'Hara, Vicky Monsalve, Hèléne Côté, Jacquie Quandt, Karuna Karunakaran, Maria Issa, Amal El-Naggar, Hanh Huynh, Brian Skinnider, Hamid Masoudi, Linda Hoang, Aleksandra Stefanovic, Will Lockwood, Iva Kulic, Mike Nimmo, Tony Ng, Nickolas Myles, Muhammad Morshed, Dechang Yang and Wan Lam.

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

We hope you enjoy Pathology Day 2015.

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

A Message from the Head

Michael Allard, BSc, MD, FRCP(C)
Professor and Head
Department of Pathology and Laboratory Medicine
Faculty of Medicine
University of British Columbia

Pathology Day is a critically significant event in the departmental calendar as it serves as a time to showcase the broad spectrum of scholarly activities performed by our trainees and, by extension, our faculty. This gathering also provides a perfect venue to recognize outstanding contributions by members of the department. Pathology Day serves another very important function as well. It is an opportunity to get together to socialize and learn more about one another as well as gain an appreciation and understanding for the breadth of scholarly activities that take place in our geographically dispersed department. As for last year, a few members of our department will present a brief overview of their work as a way to facilitate this appreciation and understanding. We are very fortunate to have two outstanding individuals participate in the program this year, highlighting academic excellence and continuing in the tradition of having world leaders in their disciplines speak at Pathology Day. Dr. Don Brooks will give the James Hogg Lecture, while Dr. Brett Finlay (Professor of Biochemisty and Molecular Biology and Microbiology and Immunology, UBC) is our Keynote Speaker.

We have also chosen the top Postdoctoral Fellow abstract for platform presentation, Dr. Hugo Horlings (Dr. David Huntsman's lab). I wish to extend my sincere thanks and gratitude to members of the committee responsible for organizing the event, including Dr. Avi Ostry, Dr. Corree Laule, Zunaira Saleem, and Adeline Chan, as well as all the other individuals whose efforts make the day a success.

Hoping you enjoy this year's Pathology Day!

Rm. G227, 2211 Wesbrook Mall
Vancouver, BC

www.pathology.ubc.ca
dbertanjoli@pathology.ubc.ca

KEYNOTE LECTURE

THE ROLE OF THE MICROBIOTA IN ASTHMA

B. BRETT FINLAY, PHD

Professor in the Michael Smith Laboratories, and the Departments of Biochemistry and Molecular Biology, and Microbiology and Immunology at the University of British olumbia

Short Biography:

Dr. B. Brett Finlay is a Professor in the Michael Smith Laboratories, and the Departments of Biochemistry and Molecular Biology, and Microbiology and Immunology at the University of British Columbia. He obtained a B.Sc. (Honors) in Biochemistry at the University of Alberta, where he also did his Ph.D. (1986) in Biochemistry under Dr. William Paranchych, studying F-like plasmid conjugation. His post-doctoral studies were performed with Dr. Stanley Falkow at the Department of Medical Microbiology and Immunology at Stanford University School of Medicine, where he studied Salmonella invasion into host cells.

In 1989, he joined UBC as an Assistant Professor in the Biotechnology Laboratory. Dr. Finlay's research interests are focussed on host-pathogen interactions, at the molecular level. By combining cell biology with microbiology, he has been at the forefront of the emerging field called Cellular Microbiology, making several fundamental discoveries in this field, and publishing over 460 papers (h index=84).

His laboratory studies several pathogenic bacteria, with Salmonella and pathogenic E. coli interactions with host cells being the primary focus. He is well recognized internationally for his work, and has won several prestigious awards including the E.W.R. Steacie Prize, the CSM Fisher Scientific Award, CSM Roche Award, a MRC Scientist, five Howard Hughes International Research Scholar Awards, a CIHR Distinguished Investigator, BC Biotech Innovation Award, the Michael Smith Health Research Prize, the IDSA Squibb award, the Jacob Biely Prize, the Canadian Killam Health Sciences Prize, the Flavelle Medal of the Royal Society, the Queen Elizabeth II Diamond Jubilee Medal, the Prix Galien, is a Fellow of the Royal Society of Canada and the Canadian Academy of Health Sciences, is a Member of the German National Academy of Sciences, American Academy of Microbiology, Chair d'État, Collège de France and is the UBC Peter Wall Distinguished Professor.

He is an Officer of the Order of Canada and Order of British Columbia. He is a cofounder of Inimex Pharmaceuticals, Inc., scientific cofounder of Vedanta Pharmaceuticals, Director of the SARS Accelerated Vaccine Initiative, and Founding Director and Senior Fellow of CIFAR's Microbes and Humans.

He also serves on several editorial and advisory boards, and is a strong supporter of communicating science to the public.

JAMES HOGG LECTURE

"WHAT'S POLYMER SCIENCE DOING IN A NICE PLACE LIKE THE CENTRE FOR BLOOD RESEARCH?"

DONALD E. BROOKS, PHD

Professor of Pathology and Chemistry, Special Advisor on External Relations, Research Office of the Vice-President Research & International

Short Biography:

Dr. Don Brooks is a Physical Biochemist and Professor in the Departments of Pathology & Laboratory Medicine and Chemistry and Special Advisor on External Relations, Research to the UBC VP Research Office. Following a variety of service roles in Pathology he served as UBC's Associate Vice President Research & International from 2001 to 2011 and as Founding Director of the SPARC Office 2011-2013. In these positions he played a leadership role in promoting research (federally and provincially), building research excellence, capacity and competitiveness at U.B.C.

His major current research interests are in surface and polymer chemistry, particularly in developing polymer constructs for biomedical applications including blood compatible materials, blood plasma protein substitutes and drug delivery in which his group is widely recognized. He was one of the five faculty who founded and were awarded CFI funding to build the UBC Centre for Blood Research. He has over 150 research publications, has edited four books and holds four issued U.S. patents.

He received the 2000 UBC Alumni Award for Research, a 2002 Golden Jubilee Medal, is a Fellow of the Canadian Academy of Health Sciences, has been recognized by NASA and the American Chemical Society for his research and was presented with the 'Golden Bow Tie' David Hardwick Lifetime Achievement Award by Pathology in 2014.

GUEST SPEAKERS

DAVID GRANVILLE, PHD

Professor, Department of Pathology and Laboratory Medicine, UBC

CHERYL WELLINGTON, PHD

Professor, Department of Pathology and Laboratory Medicine, UBC

HUGO HORLINGS, MD, PHD

Postdoctoral Fellow, BC Cancer Agency · Pathology (Dr. David Huntsman's lab)

CONFERENCE OUTLINE

Cordula and Gunter Paetzold Health Education Centre, 1st floor, Jim Pattison Pavillion North, Vancouver General Hospital

7:45 am	Breakfast
7:55 am	Opening remarks – Dr. Michael Allard
8:00 am – 9:00 am	**James Hogg Lecture** in the Paetzold Lecture Theatre: Dr. Don Brooks *"What's polymer science doing in a nice place like the Centre for Blood Research?"*
9:00 am – 9:20 am	**Guest speaker**: Dr. David Granville in the Paetzold Lecture Theatre *"Granzymes in Aging and Impaired Wound Healing"*

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)	
9:25 am – 9:40 am	Tissa Rahim
9:40 am – 9:55 am	Katerina Othonos
9:55 am – 10:10 am	Anthony Hsieh
10:10 am – 10:25 am	Abhinav Ajaykumar

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)	
9:25 am – 9:40 am	Gang Wang (AP -3)
9:40 am – 9:55 am	Noorah Almadani (AP-3)
9:55 am – 10:10 am	Brandon Sheffield (AP-4)
10:10 am – 10:25 am	Maziar Riazy (AP-3)

10:25 am – 10:40 am	Break (Atrium)
10:40 am – 11:00 am	**Guest speaker:** Dr. Cheryl Wellington in the Paetzold Lecture Theatre *"Alzheimer's Disease and Traumatic Brain Injury"*

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)	
11:05 am – 11:20 am	Hani Bagheri
11:20 am – 11:35 am	Anja Mottok
11:35 am – 11:50 am	Gabriel Fung

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)	
11:05 am – 11:20 am	Lawrence Lee (AP-3)
11:20 am – 11:35 am	Tyler Hickey (AP-2)
11:35 am – 11:50 am	Tyler Verdun (GP-4)
11:50 am – 12:05 am	Shazia Masud (MM-3)

MSAC: The William A. Webber Medical Student & Alumni Centre is located on the corner of 12th Avenue and Heather Street, near Vancouver General Hospital.

12:15 pm – 2:30 pm	Poster Session & Lunch at Medical Student Alumni Centre (MSAC)
2:45 pm – 3:05 pm	**Guest speaker:** Dr. Hugo Horlings, Post Doctoral Fellow (Dr. David Huntsman's Lab) in the Paetzold Lecture Theatre *"Granulosa-Cell Tumor of the Ovary: a Molecularly Defined Entity"*

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)

3:10 pm – 3:25 pm	Yulia Merkulova
3:25 pm – 3:40 pm	Linnette Ocariza
3:40 pm – 3:55 pm	Jacky Leung
3:55 pm – 4:10 pm	Jon Obst

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)

3:10 pm – 3:25 pm	Inna Sekirov (MM-3)
3:25 pm – 3:40 pm	Shadhiya Al Khan (HP-4)
3:40 pm – 3:55 pm	Natalie Blaszczyk (HP-4)
3:55 pm – 4:10 pm	Habib Razvi (HP-4)

4:30 pm – 5:30 pm	**Keynote speaker** Dr. Brent Finlay in the Paetzold Lecture Theatre *"The Role of the Microbiota in Asthma"*
5:45 pm	Cocktails and Canapés (MSAC)
6:00 pm	Awards (MSAC) evening event closes at 8:30 pm

Thank you for making Pathology Day 2015 a success!

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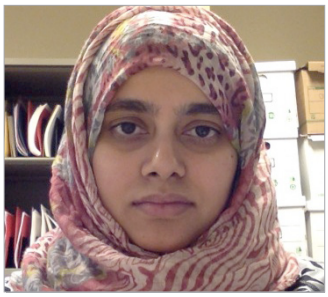


SHADHIYA AL KHAN

Supervisor: Dr. Suzanne Vercauteren
Session: Clinical Sciences

"Leukemia Associated Immunophenotype as a Diagnostic Criterion for Precursor B- Cell Lymphoblastic Leukemia"

AUTHORS:
Habib M. Razavi[1], **Shadhyia Al Khan[1]**, Angela Tsang[2], Caron Strahlendorf [3,4], Sheila Pritchard[3], and Suzanne Vercauteren[1]

AFFILIATIONS:
Department of Pathology and Laboratory Medicine, University of British Columbia;Division of Hematopathology BC Children's Hospital; Division of Hematology and Oncology BC Children's Hospital; Child and Family Research Institute

Background/objectives:
Precursor B- Acute Lymphoblastic Leukemia is the most common malignancy in childhood. Apart from abnormal morphology blast cells often express an aberrant immunophenotype (leukemia-associated immunophenotype or LAIP), which can be assessed by minimal residual disease type flow-cytometry. Here we report two pediatric cases in whom we used LAIP to show a leukemic clone. We propose that LAIP by flow cytometry is a unique tool for diagnosing evolving leukemia before a morphological diagnosis of leukemia (>20% blasts in blood or bone marrow) could be made.

Methods:
We present two pediatric cases where the diagnostic criteria for B-Acute lymphoblastic leukemia (B-ALL) was not met, but an LAIP was identified. We performed flow cytometric analysis on the bone marrow aspirate samples using the minimal residual disease (MRD) panel. The panels consist of the following antibodies: Tube 1: CD45-PC5, CD19-PC7, CD20-PE, CD10-ECD and CD38-FITC and Tube 2: CD45-PC5, CD19-PC7, CD13+CD33-PE, CD10-ECD and CD34-FITC. We subsequently compared the blasts immunophenotype when both patients developed morphologically overt B-ALL.

Results:
Both patients' blasts had an abnormal LAIP consistent with precursor B lymphocytic phenotype on initial presentation. The immunophenotype was not consistent with hematogone maturation as it fell outside our established normal maturation pattern. Both patients subsequently developed B-ALL as defined by the WHO 1-3 months after the first detection of LAIP and the MRD immunophenotyping showed the exact LAIP as noted initially.

Conclusions:
The detection of a small leukemic population in patients who are symptomatic but do not meet the WHO criteria for precursor B ALL can generate a dilemma for physicians regarding whether they should be commenced on chemotherapy. Whether initiation of therapy might have been appropriate at an earlier time point needs further evaluation. We hope that provision of these cases initiates a discussion as to whether LAIP should be included in the diagnostic criteria of B-ALL. Flow cytometry can potentially allow the diagnosis and treatment of children at an earlier time point. Whether this would have prognostic consequences remains to be determined.

NOORAH ALMADANI

Supervisor: Dr. Jefferson Terry
Session: Clinical Sciences

"Frequent Loss of Mismatch Repair Proteins in Dedifferentiated Endometrial Carcinoma"

AUTHORS:
Lien N. Hoang[1], **Noorah Almadani[1]**, Basile T. Cloutier[1], Mary Kinloch[2], Martin Kobel[3], Guangming Han[4], Jessica N. McAlpine[5], David G. Huntsman[1], C. Blake Gilks[1], Cheng-Han Lee[6]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, and Genetic Pathology Evaluation Center, Vancouver General Hospital and University of British Columbia;
[2]Department of Pathology and Laboratory Medicine, Saskatoon City Hospital and University of Saskatchewan;
[3]Department of Pathology and Laboratory Medicine, Calgary Laboratory Services and University of Calgary;
[4]Department of Laboratory Medicine and Pathology, Sunnybrook Health Sciences Centre, University of Toronto;
[5]Department of Gynecology and Obstetrics, University of British Columbia;
[6]Department of Laboratory Medicine and Pathology, Royal Alexandra Hospital and University of Alberta

Background/objectives:
Dedifferentiated endometrial carcinomas (DEC) are rare endometrial tumors and are described as undifferentiated carcinoma occurring in association with a low-grade (FIGO grade 1 or 2) endometrioid carcinoma component [1,2]. The recognition of (DEC) is clinically reproducible, as they were found to have a worse prognosis compared to (FIGO) grade 3 endometrioid carcinoma, the main entity in the differential diagnosisas well as serous endometrial carcinoma [1]. Studies exploring the morphologic characteristics of endometrial carcinoma with DNA mismatch repair (MMR) protein abnormalities and/or microsatellite instability, found an association with dedifferentiated histology. Few studies, with relatively small sample size, investigated the frequencies of (MMR) protein abnormalities in (DEC). The majority of these studies showed MLH1/PMS2 protein abnormality[3,4].

Methods:
Herein, we collected a larger and multi-institutional series of (DEC).

Results:
Of the 44 cases of (DEC), we found that 31 cases (70%) had abnormal (MMR) proteins by immunohistochemical analysis. MLH& PMS2 abnormality / mutation was predominant and was identified over 70% of the cases.

Conclusions:
In summary, we found a high frequency of (MMR) protein abnormalities in (DEC). Although the correlation of these findings with Lynch Syndrome is unknown, centers without universal immunohistochemical screening for (MMR) proteins might consider screening for all cases of endometrial carcinoma with dedifferentiated histology.

NATALIA BLASZCZYK

Supervisor: Dr. S. Jackson
Session: Clinical Sciences

"Comparison of One Stage Clotting and Two Stage Chromogenic Factor VIII Assay Results and Correlation of Discrepant Results with Specific Patient Bleeding Histories"

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

AFFILIATIONS:
University of British Columbia, Faculty of Medicine, Department of Pathology and Laboratory Medicine

Background/objectives:
It is a known phenomena that 20-30% of patients with mild or moderate hemophilia A (FVIII:C 6-40% and 1-5%, respectively) demonstrate discrepant baseline factor VIII (FVIII) as measured by a one stage when compared to a two-stage PTT-based FVIII assay. Historic two stage FVIII assays have been replaced in the coagulation laboratory by chromogenic FVIII activity kits that also generate factor X as an intermediary step. When used in mild-moderate hemophilia A chromogenic FVIII assays will sometimes yield significantly lower FVIII activity than the standard one stage assay. The lifetime bleeding tendency may be more consistent with a two-stage or chromogenic assay and sometimes the severity of hemophilia A merits reclassification.

Methods:
As a pilot, FVIII activity as measured by a one stage PTT-based FVIII assay from Instrument Laboratories were compared to a chromogenic assay (biophen from Anira). A result was considered discrepant when the ratio of one stage/chromogenic was ≥2 or <0.5. We then examined the existing hemophilia severity classification and bleeding tendencies/clinical histories of the patients with discrepant assay results. Samples from patients with known inhibitors and those who had recently received treatment with FVIII concentrates were not included.

Results:
Patients with severe, moderate and mild factor VIII deficiency were examined. The severely factor VIII deficient patients were either frequently treated with or on continuous factor replacement, and thus could not be included in further analysis. Of the mild factor VIII deficient patients about half had discrepant results. Both type A and B discrepancies were seen. The patients with discrepant results that could potentially warrant reclassification of their hemophilia severity would be mostly from mild to moderate based on the chromogenic assay. Only rare patients could be reclassified as mild to normal. Of the patients with moderate deficiency, most would not be reclassified. Finally only some of the patients with "numerically" discrepant results have slightly more clinical bleeding than could be expected based on their one stage FVIII assay levels, and are more consistent with their discrepant chromogenic assay result.

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

ABSTRACT #4

TYLER HICKEY

Supervisor: Dr. Diana Ionescu
Session: Clinical Sciences

"Right Cardiac Ventricular Rupture Secondary to CPR in a Patient with Diffuse Bilateral Pulmonary Embolism"

AUTHORS:
Tyler Hickey[1,2], Gina Gill[3,4], Douglas Webber[1,2]

AFFILIATIONS:
[1]Department of Anatomical Pathology, Vancouver General Hospital; [2]Department of Pathology and Laboratory Medicine, University of British Columbia; [3]Department of Emergency Medicine, Vancouver General Hospital; [4]Department of Emergency Medicine, University of British Columbia

Background/objectives:
Cardiopulmonary resuscitation (CPR) using external chest compressions is a commonly used maneuver in the acute care setting for patients exhibiting inadequate organ perfusion secondary to cardiac dysfunction. CPR, when performed properly, is a traumatic treatment for the patient. The extent of iatrogenic trauma is variable; potentially harming a variety of tissues within neck, thorax and superior abdomen. Certain injuries are common (e.g. rib and sternal fractures), while other injuries are rare (e.g. myocardial rupture). Here we review the frequency and types of injuries associated with CPR and describe a rare case of right ventricular rupture secondary to CPR.

Methods:
Our case study examination was conducted through review of the patient records of the deceased, discussion of the sequence of events during the resuscitation with the Emergency Physician running the code, and finally, an autopsy and histologic examination of all relevant tissues related to the cause of death.

Results:
In our case, the patient exhibited several bilateral rib fractures resultant from CPR trauma. One of these rib fractures was regionally related to a pericardial tear. A 1 cm full thickness tear of the lower anterior right ventricle was also present, which was not obviously regionally related to a rib fracture. A large volume of hemorrhage was present in the mediastinum and right supraclavicular area. Examination of the lungs revealed gross and microscopic evidence of acute and chronic diffuse bilateral pulmonary embolic disease.

Conclusions:
CPR-related injuries are common. Chest wall injuries are the most frequent injury, and most CPR-related injuries can be managed without significant morbidity and mortality in the post-resuscitation period. There are very few described cases of ventricular rupture secondary to CPR. Of the right ventricular rupture cases described, approximately half occurred in patients with conditions causing increased right heart strain (e.g. pulmonary embolism). We propose diffuse pulmonary embolic disease, coupled with CPR, contributed to a rare occurrence of right ventricular rupture in this case.

LAWRENCE LEE

Supervisor: Dr. David Schaeffer
Session: Basic Sciences

"Histopathologic Grading of Appendiceal Goblet Cell Carcinoids Predicts Survival"

AUTHORS:
Lawrence Lee[1], Yarrow McConnell[2], Erica Tsang[3], Hagen Kennecke[3], Siham Zerhouni[2], David F. Schaeffer[1]

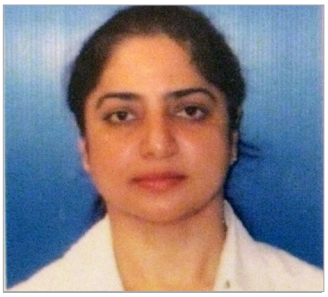
AFFILIATIONS:
[1]Division of Anatomic Pathology, Vancouver General Hospital; [2]Surgical Oncology, Division of General Surgery, Vancouver General Hospital; [3]Division of Medical Oncology, BC Cancer Agency

Background/objectives:
Goblet cell carcinoid (GCC) is a rare appendiceal malignancy with both neuroendocrine and glandular features. Clinical outcomes of patients with GCC vary widely and histology-based 3-tiered prognostic scheme has been previously suggested but is subjective and challenging to apply in day-to-day practice. We sought to construct a simplified and prognostic grading system based on objective histologic features with specific criteria.

Methods:
A continuous population-based cohort of GCC with clinical outcome data (n=87) was identified (median follow-up period of 35.9 months), of which 78 cases underwent detailed histopathological review. Specific histologic features, including cytologic atypia, peritumoral stromal desmoplasia, and solid growth pattern, were recorded and a scoring system was devised based on the presence of these features.

Results:
Using the scoring system, GCC were separated into low-grade (n=55, 71%) and high-grade (n=23, 29%) histologic variants. Correspondingly, clinical follow-up data shows good prognosis in those with low-grade histology with a median and 10-year overall survival of 51.0 months and 80.5%, respectively, while those with high-grade histology have poor prognosis with median and 10-year overall survival of 16.5 months ($p=0.006$) and 0% ($p<0.001$), respectively. Multivariate Cox proportional hazard model shows that TNM clincopathologic staging is a strong predictor of overall survival and further demonstrates that histologic grading predicts survival independent of stage.

Conclusions:
These data show that a simple and objective histologic scoring system separates GCC into low- and high-grade histology with divergent clinical outcomes and has the potential to guide clinical management of such patients.



SHAZIA MASUD

Supervisor: Dr. Diane Roscoe
Session: Clinical Sciences

"Evaluation of Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for Yeast Identification"

AUTHORS:
Masud Shazia[1], Roscoe Diane[1,2], Stefanovic Aleksandra[1,2], Hoang Linda[1,3]

AFFILIATIONS:
[1]Department of Pathology and laboratory Medicine, University of British; [2]Vancouver General Hospital; [3]British Columbia Public Health Microbiology and Reference Laboratory, Provincial Health Services Authority

Background/objectives:
For most yeast species, antifungal susceptibility can be predicted based on yeast identification. MALDI-TOF was applied in this study to assess its accuracy when compared to traditional phenotypic methods for rapid yeast species identification

Methods:
The microbiology database was used to identify clinical yeast isolates recovered from blood and other sterile body sites from Jan 2012 to Dec 2014. The isolates were sub-cultured twice to a BAP (Oxoid- Basingstoke, UK). Each isolate was then sub cultured to Brilliance™ Candida Agar (Oxoid- Basingstoke, UK) for presumptive identification. Final identification was confirmed using germ tube formation for C. albicans or AUXACOLOR 2 (Bio-Rad, Marnes-la-Coquette, France) for Candida non-albicans and Cryptococcus species. MALDI-TOF MS (Bruker Daltonics, Bellerica, MA) was used to analyze yeast isolates using spot plate extraction method. For isolates with lower identification scores, additional tube extraction was performed. A score value of ≥ 2 and category A was considered to be a reliable ID. In case of discrepant results, ITS sequencing was performed.

Results:
This study tested a total of 62 isolates of Candida species and six isolates of Cryptococcus species. The concordance in yeast identification between conventional phenotypic methods and MALDI-TOF was high (100% at genus level and 96%% at species level). The only discrepancy (no reliable ID by AUXCOLOR) was further tested by ITS sequencing which confirmed the MALDI-TOF identification. Two isolates of C. parapsilosis, although identified correctly by MALDI- TOF failed to achieve the reliable score values.

Yeast Species (74)	Phenotypic ID	MALDI-TOF Plate extraction ID	*MALDI-TOF Tube extraction ID	ITS Sequencing
C.albicans (29)	29	26	3	
C.glabrata (12)	12	11	1	
C.parapsilosis (12)	12	8	2	2
C.tropicalis (5)	5	3	2	
C.lusitaniae (3)	3	3	-	
C.krusei (2)	2	1	1	
C.guilliermondii (1)	none	-	1	1
C.neoformans (7)	7	7	-	
C.gattii (3)	3	1	2	
Total # of Species ID	73	60	12	3

* Tube extraction performed only for isolates failed to achieve reliable ID scores with Plate extraction

Conclusions:
Identification of yeast especially Candida species by MALDI-TOF is accurate and reliable. The rapid identification of yeast by MALDI-TOF compared to conventional methods will substantially improve fungal diagnosis and choice of correct antifungal therapy.

HABIB MOSHREF RAZAVI

Supervisor: Dr. Bakul Dalal
Session: Clinical Sciences

"Comparison of Neutrophil and Monocyte Clone Size in Patients with GPI-deficient Cells"

AUTHORS:
Habib Moshref Razavi, Bakul Dalal, Mabel Ma, Nikisha Khare, Steven Pi and Alok Vakil

AFFILIATIONS:
[1]Department of Hematological Pathology, Vancouver General Hospital, Vancouver, BC Canada

Background/objectives:
Neutrophils and RBCs are routinely tested for expression of glycosyl phosphatidyl inositol-associated antigens (GPI-testing) in paroxysmal nocturnal hemoglobinuria (PNH) and certain bone marrow failure states. However, monocytes are not routinely tested and their clinical utility is unknown. We report here comparison of results of GPI-testing in neutrophils and monocytes using high resolution flow cytometry.

Methods:
High resolution GPI-testing was done in 982 instances on 873 patients over a 3.5 years period. A 7-color panel consisting of CD45, CD15, CD33, CD64, CD14, CD24 and FLAER was used for assessment of neutrophils (gated with CD15) and monocytes (gated with CD33 and CD64), while a 2-color panel consisting of CD235 and CD59 was used for RBCs (gated with CD235). Minimum 25 GPI-deficient cells making a tight cluster in two parameter dot plot were deemed necessary to consider a specimen positive.

Results:
A positive result (i.e. presence of GPI-deficient cells) was seen in 94/982 (9.6%) tests in 42/873 (4.8%) of patients. The clone size of GPI-deficient cells varied from 0.01 to 98.00%. All the patients with positive GPI-testing in neutrophils were also positive in monocytes, and vice versa. In 56/94 positive results, the size of the monocyte clone was higher than neutrophils. Variance between the clone size of the neutrophils and monocytes ranged from -48% to +32%. The clone size is a soft criterion for the diagnosis of PNH, and is also a determinant for prophylactic anticoagulation. In 11 cases, the clone size of neutrophils and monocytes varied by >10%. Of these, two cases of PNH had a considerably larger monocyte clone that would have been clinically significant.

Conclusions:
Qualitative (positive or negative) results for GPI-testing for neutrophils and monocytes are generally synchronous. However, quantitative (clone size) results for the two cell types vary widely, and the difference may be clinically significant.

MAZIAR RIAZY

Supervisor: Dr. David F Schaeffer
Session: Clinical Sciences

"Mismatch Repair Status as a Predictive Marker in Pancreatic Ductal Adenocarcinoma"

Background/objectives:
Deficiencies in DNA mismatch repair (MMR) have been associated with inferior response to 5-FU in colorectal cancer. Pancreatic ductal adenocarcinoma (PDAC) is similarly treated with pyrimidine analogues yet the predictive value of MMR status for response to these agents has not been examined in this malignancy.

Methods:
A tissue microarray with associated clinical outcome, comprising 254 resected PDAC patients was stained for four MMR proteins (MLH1, MSH2, MSH6 and PMS2). MMR-deficiency and MMR proficiency was determined by the absence or presence of uniform nuclear staining in tumor cells, respectively. Univariable and multivariable survival were assessed.

Results:
Of the 254 cases, 79 (31%) received adjuvant treatment with a pyrimidine analogue and 45 (18%) showed a MMR-deficient immunoprofile. In the MMR-proficient cohort, adjuvant chemotherapy and regional lymph node status were significant independent prognostic variables of disease specific survival (DSS) ($p<0.0001$); however, no difference existed between the MMR-deficient and proficient cohorts with regard to these variables. Univariable assessment of DSS revealed an almost identical survival profile for both treated and untreated patients with a MMR-deficient profile, while treatment in the MMR-proficient cohort conferred a greater than 1-year median DSS advantage over their untreated counterparts ($p<0.0001$). In our cohort, adjuvant chemotherapy with a pyrimidine analogue conferred no survival advantage to MMR-deficient PDAC patients.

Conclusions:
Pending validation of these findings in specimens from previously conducted randomized trials with an untreated control arm, MMR immunoprofiling can be used as a feasible predictive marker in PDAC patients.

AUTHORS:
Maziar Riazy[1,2], Steve E. Kalloger[2,3], Brandon S. Sheffield[1,2], Renata D. Peixoto[4], Hector H. Li-Chang[1,2], Charles H. Scudamore[2,3,5], Daniel J. Renouf[2,3,4], David F. Schaeffer[1,2,3]

AFFILIATIONS:
[1]Division of Anatomic Pathology, Vancouver General Hospital; [2]The University of British Columbia; [3]Pancreas Centre BC; [4]Division of Medical Oncology, BC Cancer Agency; 5 Division of General Surgery, Vancouver General Hospital

INNA SEKIROV

Supervisor: Dr. Linda Hoang
Session: Clinical Sciences

"Carbapenemase Producing Organisms in British Columbia, 2008-2014"

AUTHORS:
Inna Sekirov[1,2], Corrinne Ng[1], Robert Azana[1], Yin Chang[1], Matthew Croxen[1], Benjamin Mack[2,3], Manal Tadros[2,3], Elizabeth Brodkin[2,3], Pamela Kibsey[4], Aleksandra Stefanovic[2,5], Sylvie Champagne[2,6], Edith Blondel-Hill[2,7], Michael Mulvey[8], Linda Hoang[1,2]

AFFILIATIONS:
[1]British Columbia Public Health Microbiology and Reference Laboratory, Provincial Health Services Authority; [2]Department of Pathology and Laboratory Medicine, University of British Columbia; [3]Surrey Memorial Hospital, Fraser Health Authority; [4]Victoria General Hospital, Vancouver Island Health Authority; [5]Vancouver General Hospital, Vancouver Coastal Health; [6]St. Paul's Hospital, Providence Health Care; [7]Kelowna General Hospital, Interior Health Authority; [8]Public Health Agency of Canada

Background/objectives:
Carbapenemase-producing organisms (CPOs) are a serious emerging problem for health care facilities world-wide. Owing to their resistance to most antimicrobial therapies, CPOs are difficult to treat and pose a challenge for infection control and prevention. Since 2010, province-wide phenotypic screening and PCR-based testing for CPOs were implemented in British Columbia (BC), Canada. A review of CPOs in BC, from 2008 to March 2014, was done with the objective to characterize resistance mechanisms, possible person-to-person transmission and potential institutional spread.

Methods:
During this study period, a total of 177 cases were identified. Data on pertinent patient demographics and resistance mechanisms genetically encoded by the isolates, as well as antibiogram profiles were reviewed and descriptive analysis of their numbers and resistance mechanisms was carried out. Pulsed-field gel electrophoresis (PFGE) profiles for a subset of E. coli, K. pneumoniae and Enterobacter spp. isolates were obtained and analysed using the BioNumerics software.

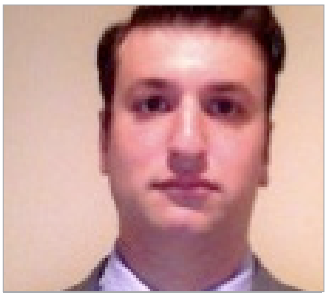
Results:
Our findings demonstrate that CPOs have been increasing in numbers in BC over time, from 1 isolate/year in 2008 and 2009, to 82 isolates in 2013 and 30 isolates in only the first quarter of 2014. The increasing numbers are in big part due to enhanced surveillance for CPOs, as the majority of isolates are from surveillance rather than clinical samples. K. pneumoniae isolates overall lack clonality, although apparently related clusters are found, especially in the OXA-48 carbapenemase-producing subset. Analysis of Enterobacter spp. isolates revealed a more clonal nature of these CPOs in BC

Conclusions:
Rising numbers of CPOs demonstrate the need for ongoing tight infection control practices. Presence of apparently related clusters of organisms argues towards evidence of inter-patient organism transmission both within and across institutions. Additionally, there is a possibility of transmission of mobile elements between organisms, which needs to be further addressed.

BRANDON SHEFFIELD

Supervisor: Dr. Torsten Nielsen
Session: Clinical Sciences

"Molecular Profiling of ER Weakly-Positive Breast Cancer"



Background/objectives:
The estrogen receptor (ER) is a key predictive biomarker in the treatment of breast cancer. Luminal subtypes of breast cancer express ESR1, and are eligible for hormonal therapy. Standard laboratory assessment of ER status is currently immunohistochemistry (IHC)-based. Since the introduction of ER IHC, the threshold for positivity has decreased from 10% to > 1% of tumor cells whilst methodological advances have increased the sensitivity of IHC detection. These trends have led to considerable uncertainty regarding the use of hormonal therapy in the setting of ER weakly-positive tumors. We report intrinsic subtype classification on a cohort of ER weakly-positive early stage cancers and discuss the implications on clinical treatment.

Methods:
Consecutive cases of untreated, surgically resected primary breast cancer were retrospectively identified from 4 tertiary care centres in British Columbia, Canada. All participating centres engage in routine external proficiency testing for breast biomarkers. Based upon the semiquantitative Allred score, combining staining intensity and percentage of tumor cells, ER-negative (Allred 0 and 2) and ER weakly-positive (Allred 3-5) cases were included. Gene expression profiling was performed using qRT-PCR. Intrinsic subtype prediction was made based upon the PAM50 gene expression signature.

Results:
153 cases were included in the series; 62 cases originally diagnosed as ER weakly-positive and 91 ER-negative. Weak ER signal by IHC correlated poorly with ESR1 gene expression by qRT-PCR (R2 =0.2 [0.1-0.3 95% CI]). Of the 62 cases originally assessed as ER weakly-positive, only 6 (10%) were confirmed as luminal subtype by gene expression profiling with the remaining cases showing basal-like or HER2-enriched subtypes (90%). In this highly enriched ER weak-positive cohort, the positive predictive value of low-level ER staining for luminal subtype was extremely poor (8% [3%-18% 95%CI]).

Conclusions:
Weak ER expression by IHC is a poor correlate of both luminal subtype and ESR1 expression in invasive breast cancer. In the setting of highly sensitive and robust IHC methodology, cutoffs for ER status determination and subsequent systemic therapy may need to be revisited.

AUTHORS:
Sheffield BS[1], Kos Z[2], Wang X[1], Chow C[3], Lau S[3], Wolber R[4], Gilks CB[1,3,5], Myles N[1,6], Thomson T[1,7], Hayes MM[1,7], Rachamadugu R[8], Stijleman I[8], Bernard PS[8], Nielsen TO[1,3,5], Chia SKL[1,9]

AFFILIATIONS:
[1]University of British Columbia, Department of Laboratory Medicine and Pathology; [2]The Ottawa Hospital, Department of Pathology and Laboratory Medicine; [3]University of British Columbia, Genetic Pathology Evaluation Centre; [4]Lions Gate Hospital, Department of Laboratory Medicine and Pathology; [5]Vancouver General Hospital, Department of Laboratory Medicine and Pathology; [6]St. Paul's Hospital, Department of Laboratory Medicine and Pathology; [7]British Columbia Cancer Agency, Department of Laboratory Medicine; [8]University of Utah/Huntsman Cancer Center, Department of Pathology; [9]British Columbia Cancer Agency, Department of Medical Oncology

TYLER VERDUN

Supervisor: Dr. Andrew Churg
Session: Basic Sciences

"The Prognostic Significance of Tumor Subtype in Poorly Differentiated Non-small Cell Carcinomas of the Lung"

AUTHORS:
Tyler Verdun[1], Susanna Zachara[1], Cheryl Ho[1,2], Janessa Laskin[1,2], Andrew Churg[1,2]

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

Background/objectives:
Advancements in molecular oncology and the recent development of epidermal growth factor receptor tyrosine kinase inhibitors have necessitated the proper classification of non-small cell lung cancers (NSCLC) to ensure an appropriate standard of patient care. However, a significant proportion of NSCLCs show no obvious morphologic differentiation and hence cannot be diagnostically classified as squamous cell carcinoma (SCC) or adenocarcinoma (AC) by histology alone. The use of immunohistochemistry is often required to properly classify such cases. The prognostic significance of tumor subtype in high grade malignancies of the lung has yet to be elucidated.

Methods:
Cases of NSCLC diagnosed between 2009 and 2013 that were unclassifiable or ambiguous via conventional H&E histology were selected from the archives of Vancouver General Hospital and reviewed. Both transbronchial biopsy and lung resection specimens were included. Immunohistochemical stains for napsin A, TTF-1, p40/p63, and CK5/6 were used to classify each malignancy as SCC, AC, or NSCLC-NOS/large cell/undifferentiated carcinoma (LCLC). Cases showing definitive histologic features of differentiation, small cell carcinoma, or immunohistochemical profiles of NSCLC-NOS/LCLC were excluded from the study. Survival analysis by stage was performed using the Kaplan Meier method. The International Association for the Study of Lung Cancer 2009 staging dataset was used as a comparator.

Results:
A total of 254 cases were reviewed, 82 of which met the inclusion criteria. Immunohistochemical staining classified 41 cases of both AC and SCC. Survival analysis by stage demonstrated no association of specific differentiation or lack of differentiation with respect to prognosis. Cases of poorly differentiated SCC and AC of the lung show similar stage-for-stage survival, both when compared to each other and to a general cohort of lung cancer patients.

Conclusions:
A sizable minority of poorly differentiated carcinomas of the lung cannot be properly subtyped by histology alone. Immunohistochemistry is often required to properly classify such cases. However, such poorly differentiated lung carcinomas do not show any significant differences in stage-for-stage survival with respect to either tumor subtype or the general lung cancer patient population as a whole.

GANG WANG

Supervisor: Dr. Davide Salina
Session: Basic Sciences

"Proximal Epithelioid Sarcoma Following a History of Malignant Melanoma - A Diagnostic Challenge"

Epithelioid sarcoma is an uncommon soft-tissue sarcoma typically presenting as a subcutaneous or deep dermal mass in the distal extremities of young adults. Its 'proximal' subtype, which occurs in the pelvic and genital areas of somewhat older individuals, behaves more aggressively. The correct diagnosis is essential, since this tumor can be easily mistaken for malignant melanoma, with significant treatment implications. We report a 37-year-old female with a fusiform mass in the medial adductor muscles of the thigh, complicated by history of locoregional pT3bN1 malignant melanoma of the skin of her right inner thigh. Fine needle aspiration of the mass showed numerous single isolated malignant epithelioid cells containing enlarged and pleomorphic eccentric nuclei, coarse chromatin, small prominent nucleoli and dense cytoplasm with few cells displaying a rhabdoid appearance.

There was no obvious pigment, intranuclear inclusions, large massive nucleoli or binucleated cells. Core biopsy showed the tumor consisting of diffuse sheets of epithelioid cells with focal rhabdoid and spindled morphology. The cells expressed cytokeratin AE1/AE3, EMA, CD31 and Vimentin, did not express S-100, Melan A, HMB-45 or Sox-10. There was complete loss of nuclear INI1 protein expression, which confirmed the diagnosis of epithelioid sarcoma. This is the first report of diagnosing a proximal epithelioid sarcoma by FNA and core in a site of previously resected malignant melanoma. We present this case to emphasize the importance of keeping this tumor in the differential diagnosis in this sort of clinical presentation.

AUTHORS:
Gang Wang[1], Martin Hyrcza[1], Nicholas van der Westhuizen[1,2], Torsten Nielsen[1,3], and Davide Salina[1,2]*

AFFILIATIONS:
[1]Department of Pathology & Laboratory Medicine, University of British Columbia; [2]Department of Pathology, Royal Jubilee Hospital, Victoria; [3]Department of Pathology, British Columbia Cancer Agency

TISSA RAHIM

Supervisor: Dr. Jaqueline Quandt
Session: Basic Sciences

"ARNT2, a Neuroprotective Transcription Factor, as a Regulator of Neurodegenerative Processes in Models of Multiple Sclerosis"

AUTHORS:
Tissa Rahim[1], Andrew Leung[1], Adam Yu[1], Jacqueline Quandt1

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine

Background/objectives:
Multiple sclerosis (MS), the most common neurological disease of young adults in Canada, is a chronic inflammatory neurodegenerative disease of the central nervous system, causing loss of balance, impaired vision/speech and paralysis. MS is characterized by blood-brain barrier alterations, inflammatory cell infiltration, demyelination, axonal damage/loss. The most effective MS treatments targeting immune cells can be associated with risks. Therefore, research to develop therapeutic targets affording neuroprotection remains crucial. ARNT2 (aryl-hydrocarbon receptor nuclear translocator 2) is a transcription factor expressed in neurons with neuroprotective properties, specifically during ischemia. However, its role in chronic inflammatory/neurodegenerative disease has not yet been characterized. Our objective was to examine ARNT2 expression in cortical neurons following stimulation with mediators that underlie MS disease processes. We hypothesized that ARNT2 levels are influenced by several insults which alter cell viability/function, and chose to study oxidative damage, glutamate excitotoxicity and apoptosis.

Methods:
Embryonic (E16-18) neuronal cultures were treated with hydrogen peroxide (H2O2; to mimic oxidative stress), glutamate or staurosporine (a protein kinase inhibitor with apoptotic properties) to examine their influence on ARNT2 expression via western blotting and immunocytochemistry. H2O2 was tested at 25-300μM, glutamate doses ranged from 5-500μM, and staurosporine was tested at 5nM-1μM. All treatments were followed for up to 24h. Cell viability was assessed by morphological analysis/lactate dehydrogenase (LDH) release.

Results:
With all doses of H2O2 tested ARNT2 levels increased up to 30% within 30min exposure compared to control. With 100μM and 300μM H2O2, ARNT2 levels continued to decrease gradually, preceding increased LDH release/cell death by 24h. Our glutamate excitotoxicity data revealed that within 1h of 500μM glutamate exposure, ARNT2 levels were reduced by 30% and correlated with axonal/dendritic retraction. At 24h glutamate exposure, ARNT2 expression declined to 50% of control, associated with cell toxicity/death. A mild apoptotic stimulus of 10nM staurosporine increased ARNT2 levels by 75% compared to control at 24h. At an apoptotic dose of 1μM, cell death became apparent, and was associated with ARNT2 levels comparable to control. Furthermore, examining ARNT2 expression in individual neurons revealed a heterogeneous distribution, with most neurons having low expression under healthy conditions.

Conclusions:
This work suggests that ARNT2 expression is upregulated under certain stress-initiating stimuli, including mild oxidative stress, glutamate excitotoxicity and apoptotic signals, and may contribute to protective responses. Inversely, a decline in ARNT2 expression is associated with deteriorating cell health/death with higher levels of oxidative stress and glutamate excitotoxicity. Our findings will characterize the role of ARNT2 as a neuroprotective protein or a marker of cell viability in inflammatory/neurodegenerative processes, warranting consideration in the development of MS therapies.

KATERINA MARIA OTHONOS

Supervisor: Dr. Jacqueline Quandt
Session: Basic Sciences

"Exciting vs Stressing Neurons: Protective Functions of NPAS4 in Neuroinflammation and Degenerative Processes"

AUTHORS:
Katerina Othonos[1], Sarah Neil[2], Andrew Leung[2], Jacqueline Quandt[2]

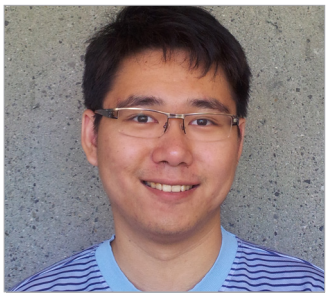
AFFILIATIONS:
[1]Graduate Program in Neuroscience, University of British Columbia;
[2]Department of Pathology and Laboratory Medicine, University of British Columbia

Background/objectives:
Multiple Sclerosis (MS) is the most common inflammatory neurodegenerative central nervous system (CNS) disorder affecting young Canadian adults. The cause of MS remains unknown; however, the demyelination and axonal loss associated with disability are believed to be driven by infiltration of various immune cells and their mediators. Neuronal Per Arnt Sim 4 (NPAS4) is a basic helix-loop-helix transcription factor that regulates genes important for neuronal survival, inhibitory synapse development and synaptic plasticity. We have recently shown that NPAS4 levels are significantly altered through the initiation and progression of experimental autoimmune encephalomyelitis (EAE), the autoimmune murine model of MS. Our findings suggest a possible immunomodulatory and neuroprotective role for NPAS4; thus, we hypothesize that changes in NPAS4 levels influence MS disease progression. The objective of this study is to examine the functional relevance of NPAS4 expression in disease development and under the influence of inflammatory cells and mediators.

Methods:
Primary cortical-enriched neuronal cultures were exposed to staurosporine (growth factor inhibitor), oxidative stress, glutamate, or co-cultured with activated immune cells to model an inflammatory setting. NPAS4 levels were determined via western blotting and immunocytochemistry; changes in cell viability were assessed by morphological analysis and lactate dehydrogenase (LDH) release. B6 mice were immunized to develop autoimmune demyelination and qPCR was performed with brain/spinal cord RNA at various time points.

Results:
Preliminary qPCR data showed NPAS4 RNA expression in the brain is increased in preclinical stages (d7) of chronic EAE compared to healthy controls and returns to normal levels by disease onset/peak (d14-18). In the spinal cord, however, following a slight increase in NPAS4 RNA expression preclinically, a steady decrease to negligible amounts occurs through onset/progression of disease when inflammation is greatest. Cortical neurons express negligible levels of NPAS4 and consistent with immune cell involvement, we observed early induction of neuronal NPAS4 expression following co-culture with splenocytes. Notably, low levels of immune cell mediators, such as glutamate and hydrogen peroxide, also rapidly increased NPAS4 expression in a time and dose dependent manner. Exposure to a range of staurosporine doses for up to 24h did not drive NPAS4 expression, despite significant cell death/LDH release.

Conclusions:
Our data suggests that NPAS4 may provide protection under normal physiological conditions and increase to respond to low stress stimuli; however its loss in tissues compared to controls can be associated with axonal loss and disability. Furthering our understanding of processes that govern CNS inflammation and neurodegeneration is important in developing efficient MS therapies that focus on limiting disease progression through neuroprotection. Our study describes novel findings related to NPAS4 regulation under inflammatory settings and suggests an important role for NPAS4 in protecting neurons throughout MS disease development.



ANTHONY HSIEH

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Chronic Human Immunodeficiency Virus Infection is Associated with Shorter Telomere in Proliferative CD8⁺ T cells"

AUTHORS:
Anthony Y.Y. Hsieh[1,2], Beheroze Sattha[1], Hélène C.F. Côté[1,2] and the CIHR team grant for the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA cohort)

AFFILIATIONS:
[1]University of British Columbia, Department of Pathology & Laboratory Medicine; [2]Centre for Blood Research

Background/objectives:
HIV Combination AntiRetroviral Therapy (cART) is highly effective in extending life expectancy and preventing transition to AIDS. However, cART-treated individuals are still at a higher risk of premature age-related comorbidities including cardiovascular disease, liver disease, non-AIDS cancers, and frailty. Putative explanations for this include immunologic abnormalities such as low level chronic inflammation and hastened lymphocyte immunosenescence that may both compromise the immune system earlier in life. Data from individuals with untreated HIV have shown an inflated senescent $CD8^+$ T cell compartment and chronic inflammation. In cART-treated infection, previous research have demonstrated decreased $CD4^+/CD8^+$ T cell ratio, reduced T cell repertoire, reduced responsiveness to vaccines, and shorter leukocyte telomere length (TL). Little is known about the molecular mechanisms behind these immune abnormalities. Our objective was to characterize TL, a molecular marker of aging, in isolated $CD4^+$ and $CD8^+$ immune compartments from HIV^+ individuals, to explore the possible role of HIV/cART on modulating immune aging. We hypothesized that HIV infection and factors such as HIV plasma viral load, time since diagnosis, or number of chronic coinfections would be associated with TL.

Methods:
In this pilot study, live PBMCs were obtained from 33 HIV^+ subjects and 10 HIV^- controls enrolled in the CARMA cohort study. FACS was used to isolate $CD4^+$, proliferative $CD8^+CD28^+$, and senescent $CD8^+CD28^-$ T cells. Relative TL was measured in all cell subsets with sufficient cell count using qPCR. Results were compared using Spearman's correlation, Mann-Whitney tests, Kruskal Wallis tests, and ANCOVA.

Results:
Consistent with previous research, the HIV^+ group presented an expanded senescent $CD8^+CD28^-$ compartment (n=33, median[IQR] 39[21-55] vs n=10, 17[6-22]% of total T cells, p=0.02) and a decreased $CD4^+/CD8^+$ ratio (n=33, 0.24[0.15-0.73] vs. n=10, 1.75[0.67-2.65], p<0.01) compared to the HIV^- group. In the entire cohort, older age was associated with shorter TL in $CD4^+$ (n=26, r_s=-0.45, p=0.02), and proliferative $CD8^+CD28^+$ (n=27, r_s=-0.47, p=0.01) but not senescent $CD8^+CD28^-$ cells (r_s=-0.06). Similarly, HIV infection was associated with shorter TL in proliferative $CD8^+CD28^+$ T cells (n=19, 3.35[2.63-3.93] vs. n=8, 3.73[3.48-4.08], p=0.02), but not in senescent $CD8^+CD28^-$ or $CD4^+$ T cells after adjusting for age. $CD4^+$ T cell compartment size was also associated with the number of chronic viral infections (HIV, hepatitis C virus or hepatitis B virus) in lifetime (n=31, p<0.01). No relationship was seen between TL and current HIV viral load, time since diagnosis, or cART in these compartments.

Conclusions:
These results suggest that HIV infection and older age may both predict shorter TL in proliferative $CD8^+$ T cells, highlighting the relevance of this compartment in studies of HIV-mediated immunosenescence. A potential cumulative effect of chronic viral coinfections on $CD4^+$ T cell compartment size may reflect the negative effect of viral burden and chronic immune activation on immune aging.

ABHINAV AJAYKUMAR

Supervisor: Dr. Hélène Côté
Session: Clinical Sciences

"Leukocyte Telomere Length (LTL) Dynamics in HIV-exposed Uninfected (HEU) Children Exposed to Combination Antiretroviral Therapy (cART) in-utero: A Cohort Study"

AUTHORS:
Abhinav Ajaykumar[1], Hugo Soudeyns[4,5], Fatima Kakkar[5], Jason Brophy[6], Ari Bitnun[7], Ariane Alimenti[2,8], Deborah M. Money[2,9,10], Arianne Y.K. Albert[2,9], Hélène C.F. Côté[1,2,3,9] and the CIHR Team on Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]BC Women's Hospital and Health Centre; [3]Centre for Blood Research, University of British Columbia; [4]Centre de Recherche du CHU Sainte-Justine, Department of Microbiology, Infectiology and Immunology, Université de Montréal, Quebec; [5]Department of Pediatrics, CHU Sainte-Justine, Université de Montréal, Quebec; [6]Department of Pediatrics, Children's Hospital of Eastern Ontario, University of Ottawa; [7]Department of Pediatrics, Hospital for Sick Children, University of Toronto; [8]Department of Pediatrics, University of British Columbia; [9]Women's Health Research Institute; [10]Department of Obstetrics, University of British Columbia

Background/objectives:
Almost half of HIV+ people are women, and approximately 10% are children. Among HIV+ children, as many as 90% get the virus from their mothers. Current combination antiretroviral therapies (cART) during pregnancy along with six weeks infant prophylaxis have been highly successful in preventing mother-to-child-transmission in the 1.5 million children born to HIV+ mothers each year. However, exposure to maternal cART during pregnancy could have long-term consequences for HEU children. Some antiretrovirals and HIV proteins inhibit telomerase, the enzyme that elongates telomeres. Leukocyte telomere length (LTL) is a marker of cellular aging and has been linked to numerous age-related morbidities such as cardiovascular diseases, neurodegenerative diseases, frailty etc. Our objective was to evaluate the impact of perinatal HIV and cART exposure on children's LTL by comparing a cohort of HEU and HIV-unexposed uninfected (HUU) control children at birth, over the first 3-6 weeks of life prophylaxis period, and over the first three years of age.

Methods:
Relative LTL was measured by qPCR in 324 HEU (0-3y, of whom 214 had ≥2 blood samples collected), as well as 308 HUU children (0-3y, single blood sample each). Univariable analyses were peformed to investigate the association between LTL at birth and the following explanatory variables: infant sex, gestational age (GA) at birth, infant birth weight, small for gestational age (SGA, yes vs. no), maternal age at birth, ethnicity (Aboriginal, Black, Asian and others vs. White), and smoking ever during pregnancy (yes vs. no). Important factors ($p<0.15$) were investigated using multivariable linear regression modeling. Longitudinal LTL in HEU between 0-3y was analyzed via a generalized mixed effects additive model.

Results:
In a cross-sectional multivariable analysis of LTL at birth (0-3d) in 115 HEU (56% male) and 91 HUU (54% male) children, male sex ($p=0.02$) and higher infant birth weight ($p=0.05$) were associated with shorter LTL. High smoking ever in pregnancy rates were observed in this cohort, with 56% and 43% of HEU and HUU mothers smoking respectively. Smoking in pregnancy was associated with shorter LTL in HEU but longer LTL in HUU, indicating a significant smoking*HEU/HUU interaction ($p<0.001$). Among HEU, neither duration of cART exposure in utero nor type of cART was related to birth LTL. Overall, LTL slopes for the first six weeks were positive in both groups. In HEU, this was followed by rapid LTL decline to ~1y of age, then a leveling out. Although a similar model could not be built for HUU, LTL attrition rates were similar in both groups in an age and sex-matched subset (n=214:214, $p=0.69$).

Conclusions:
This first detailed investigation of human LTL dynamics early in life suggests an initial apparent gain in LTL during the first six weeks of life, likely related to the loss of cells with short TL, followed by a rapid decline that levels off up to age three. These results further support that exposure to maternal HIV/cART in utero does not affect infant LTL, a reassuring finding. Rather, maternal smoking acts as a major modulator of infant birth LTL, likely through in utero stresses.

HANI BAGHERI

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

"Identifying the "driver" Genes for Multi-systemic 2p15-16.1 Microdeletion Syndrome Using *in vivo* Zebrafish Assays"

AUTHORS:
Hani Bagheri[1,2], Chansonette Badduke[1,2], Ying Qiao[1,2], Xianghong Shan[3], Sally Martell[1,2], Cheryl Gregory-Evans[3], Evica Rajcan-Separovic[1,2]

AFFILIATIONS:
[1]Department of Pathology & Laboratory Medicine, University of British Columbia, Vancouver; [2]Child & Family Research Institute, Vancouver, [3]Department of Ophthalmology, University of British Columbia, Vancouver

Background/objectives:
The 2p15-16.1 microdeletion syndrome (OMIM 612513) is a rare genetic disorder caused by de novo deletion of a small segment of chromosome 2 that we first described in two phenotypically similar individuals with intellectual disability (ID). So far, 23 subjects with this microdeletion have been identified world-wide (15 reported in literature and 8 new cases that we recruited) who share common clinical features including delayed neurocognitive development and consistent craniofacial defects e.g. microcephaly and facial dysmorphism. Microdeletions in the 23 subjects span ~9.8 Mb and include 37 protein-coding genes. The deletions are extremely variable in size, ranging from ~0.5 Kb to ~8 Mb with median deletion size of 3.14 Mb. This variability and the existence of non-overlapping deletions within this region complicate efforts to delineate a common critical region for this syndrome. Therefore, the causative genes for this condition remain to be elucidated.

Methods:
We used the zebrafish knock-down model to identify critical genes for the syndrome. Five candidate genes, XPO1, USP34, REL, FANCL, and VRK2 were shortlisted based on their frequency of deletion, prediction of haploinsufficiency effect (using the previously described approach [1]), cellular function, and expression in developing brain and in patient cells. The orthologues of these candidate genes in zebrafish were then targeted by RNA anti-sense oligomers called morpholinos to obtain gene knock-down in zebrafish embryos. The embryonic development was monitored in knock-downs and in controls (injected by gene-mismatch morpholinos) at 1 and 3 days post-fertilisation.

Results:
Our preliminary results suggest that the knock-down of XPO1 and REL orthologues in zebrafish results in phenotypic features similar to those observed in the human subjects. Specifically, the results suggest that a drop of ~50% of XPO1 gene expression causes abnormal growth, reduced head size and anomalous brain development in fish. Moreover, the knock-down of REL suggests abnormalities of the hindbrain, craniofacial defects and dysmorphic body in the zebrafish. The knock-down of zebrafish orthologues for USP34, FANCL, and VRK2 genes, however, did not result in any visible phenotypic abnormalities.

Conclusions:
Using zebrafish as a valuable model organism, we identified XPO1 and REL as the dosage-sensitive and the "driver" (causative) genes for the consistent phenotype observed in the patients. Our findings will set the foundation for improved understanding of their function and roles in brain development, as well as contribution to the syndrome development.

Reference: [1] Huang et al., (2010). PLoS Genet. 6: e1001154.

ANJA MOTTOK

Supervisor: Dr. Christian Steidl
Session: Basic Sciences

"Genetic Alterations of the MHC Class II Transactivator CIITA are Frequent in Primary Mediastinal Large B-Cell Lymphoma and Associated with Diminished MHC Class II Expression"

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

Methods:
We analyzed three PMBCL-derived cell lines and 45 PMBCL tissue biopsies (sequencing cohort) for the presence of mutations within the coding sequence (CDS) and the first 3kb of intron 1 using whole transcriptome paired-end sequencing (RNA-Seq), targeted re-sequencing and Sanger sequencing. We performed retroviral transduction of wild type CIITA and CIITA mutants in the CIITA and HLA-DR expression-negative cell line DEV and subsequently analyzed CIITA and HLA-DR expression using qRT-PCR, western blot and flow cytometry. Furthermore, we applied immunohistochemistry (IHC) to determine expression levels of CIITA and HLA-DR in a large cohort of PMBCL cases represented on two tissue microarrays, which were also used for fluorescence in-situ hybridization (FISH) to evaluate the presence of copy number alterations or translocations affecting the CIITA locus.

Results:
All three cell lines showed biallelic CIITA aberrations consisting of missense mutations and structural genomic rearrangements. Furthermore, we identified novel fusion transcripts in two of the cell lines. In our sequencing cohort we found structural rearrangements in 15 of 41 primary cases (36.6%). Thirty two percent harboured CDS mutations, and in 21 cases (46.7%) we detected small deletions and single nucleotide variants (SNVs) in intron 1. Ectopic expression of CIITA mutants in the DEV cell line demonstrated that genomic alterations in CIITA result in decreased CIITA protein expression and in reduction of MHC class II surface expression. FISH was interpretable in 115 samples of the extension cohort with a CIITA break-apart (CIITA-ba) frequency of 33.9% (39/115). Further analyses revealed that the presence of CIITA-ba is associated with decreased CIITA protein expression (Mann-Whitney Test, P=0.025) and we demonstrate a positive correlation between protein expression of CIITA and HLA-DR (Spearman rho=0.413, P<0.0001).

Conclusions:
We show that CIITA is frequently targeted by structural genomic rearrangements, CDS mutations and intronic deletions in PMBCL cell lines and primary PMBCL tumor biopsies. Our studies demonstrate that genomic alterations in CIITA contribute to the down-regulation of MHC class II expression in malignant lymphomas and therefore represent a potent mechanism of acquired immune privilege and escape from immune surveillance.

AUTHORS:
Anja Mottok[1,2], Bruce Woolcock[1], Fong Chun Chan[1,3], Adèle Telenius[1], Elizabeth Chavez[1], Lauren Chong[1,3], Merrill Boyle[1], Susana Ben-Neriah[1], David W. Scott[1], Lisa M. Rimsza[4], Reiner Siebert[5], Randy D. Gascoyne[1,2] and Christian Steidl[1,2]

AFFILIATIONS:
[1]Centre for Lymphoid Cancer, Department of Lymphoid Cancer Research, BC Cancer Agency, Vancouver, BC, Canada; [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [3]Bioinformatics Training Program, University of British Columbia, Vancouver, BC, Canada; [4]Department of Pathology, University of Arizona, Tucson, AZ; [5]Institute of Human Genetics, Christian-Albrechts-University Kiel & University Hospital Schleswig-Holstein, Kiel, Germany

GABRIEL FUNG

Supervisor: Dr. Honglin Luo
Session: Basic Sciences

"Coxsackieviral Infection Causes Cytoplasmic Aggregation and Cleavage of TAR DNA Binding Protein-43"

AUTHORS:
Gabriel Fung[1], Junyan Shi[1], Haoyu Deng[1], Chen Wang[1], Jingchun Zhang[1], Eric Deng[1], Honglin Luo[1]

AFFILIATIONS:
[1]Centre for Heart Lung Innovation, University of British Columbia

Background/objectives:
Coxsackievirus B3 (CVB3) is a non-enveloped, positive single-stranded enterovirus that infects the heart, pancreas and liver. CVB3 infection causes myocarditis, and its sequelae, dilated cardiomyopathy (DCM). In North America, DCM accounts for approximately 20% of heart failure and sudden death in children and young adults. Recent findings have shown similar molecular pathologies between cardiomyopathies and neurodegenerative diseases. In neurodegenerative diseases such as frontotemporal dementia and amyotrophic lateral sclerosis, insoluble-cytoplasmic TAR DNA binding protein-43 (TDP-43)-aggregates are a common biomarker contributing directly to pathological disease progression. The importance of insoluble protein aggregates in viral pathogenesis has also been recognized, resembling that of neurodegenerative diseases. TDP-43 is an RNA-binding protein and plays an essential role in regulating RNA maturation including pre-mRNA splicing of cystic fibrosis transmembrane conductance regulator (CFTR) exon 9 in the brain. However, the significance of TDP-43 in CVB3-induced cardiomyopathy remains unstudied. In this study, we investigated the interplay between CVB3 and TDP-43, comparing the molecular similarities between viral-induced cardiomyopathy and neurodegenerative diseases. We hypothesize that CVB3-infection induces the formation of cytoplasmic TDP-43 aggregates in viral-induced cardiomyopathy.

Methods:
An A/J mice strain, susceptable to viral-induced cardiomyopathy, and HeLa cells were used to study the affect of CVB3 infection on TDP-43 localization and protein expression. Transfection of fluorescent-tagged TDP-43 or CVB3-proteases was performed 24 hrs prior to CVB3 infection where indicated, followed by fixation in 4% paraformaldehyde. TDP-43 localization/aggregation was visualized using a Leica SP2 AOBS confocal fluoresence miscrope. To explore the potential mechanism by which CVB3 regulates TDP-43 protein expression, TDP-43 was analyzed in CVB3-infected cells using immunoblot techniques. Furthermore, in vitro cleavage assay was performed using purified viral proteases incubated in uninfected cell lysates for 4, 8 and 16 hrs. Lastly, a mini-gene reporter construct (TG(13) T(5)) was used to determine CFTR exon 9 skipping via reverse-transcription polymerase chain reaction techniques.

Results:
Our studies demonstrated that CVB3 infection leads to redistribution of nuclear TDP-43 into cytoplasmic aggregates in a viral protease 2A dependent manner. We also found that viral protease 3C, actively cleaves TDP-43 into two respective cleavage fragments. As a consequence of cleavage, we demonstrated that the respective N-terminal cleavage fragment inhibits native TDP43 activity in the nucleus as a transcriptional regulator.

Conclusions:
These studies demonstrate that CVB3 proteases selectively target and manipulate TDP-43 localization and function. Furthermore, these data provide evidence that the pathogenesis of viral-induced cardiomyopathy and neurodegenerative diseases may be more molecularly similar than previously believed.

JON OBST

Supervisor: Dr. Marianne Sadar
Session: Basic Sciences

"Chronic Exposure to a Novel AR-NTD Inhibitor May Induce Resistance via an AR-dependent Mechanism in Prostate Cancer"

AUTHORS:
Jon Obst[1,2], Kevin Yang[1,2], Nasrin R. Mawji[1], Jean Wang[1], Amy Tien[1], and Marianne Sadar[1]

AFFILIATIONS:
[1]Department of Genome Sciences Centre, BC Cancer Agency; [2]Department of Pathology and Laboratory Medicine, University of British Columbia

Background/objectives:
Prostate cancer represents the second leading cause of cancer related mortality among North American men. The androgen receptor (AR) has long been recognized to play a crucial role in tumour maintenance and progression; therefore its inhibition has been the cornerstone of modern therapy for men who fail primary treatment. Current therapeutic options are initially effective, however resistance ultimately develops and the disease progresses to a lethal form termed castration-resistant prostate cancer (CRPC). Our lab has discovered a new class of molecules (EPI) which inhibit the AR by binding to the N-terminal domain (NTD). The NTD interacts with transcriptional regulators and its presence is absolutely necessary for a transcriptionally functional receptor. We have previously shown that EPI-002 specifically inhibits the AR - both full length and constitutively active splice variants. Here we attempt to determine if resistance to EPI-002 occurs with chronic exposure, and to characterize the potentially resistant cell line.

Methods:
The androgen sensitive human prostate cell line LNCaP was used for all experiments. A potentially resistant cell line (LNCaP-EPIR) was generated by serially passaging parental LNCaP cells once weekly in media supplemented with EPI-002 beginning in September 2012. Once the resistant line began showing growth rates which approached the control (passage >90), growth curves were generated following treatment with EPI-002 and anti-androgen enzalutamide with and without androgen stimulation. Dose response curves validated the proliferation data. A luciferase reporter assay, western blot and qRT-PCR were utilized to measure AR transcriptional activity of each cell line in response to EPI-002 and enzalutamide treatment. LNCaP-EPIR cells were used as a xenograft model to verify in vitro resistance.

Results:
LNCaP-EPIR cells treated with 25 uM EPI-002 displayed similar growth rates to vehicle treatment - both in vitro and in vivo. Conversely, parental LNCaP lines showed significant growth inhibition in response to drug treatment. Dose response curves confirmed resistance and a significant difference in cell number was observed between LNCaP-EPIR and LNCaP cells at all concentrations tested. Protein expression and luciferase reporter data indicate that chronic EPI-002 treatment continues to inhibit AR transcriptional activity in LNCaP-EPIR cells. Intriguingly, growth of LNCaP-EPIR cells was significantly inhibited by anti-androgen enzalutamide, and sensitivity was seen in the xenograft study as well. Tumours harvested from mice treated with enzalutamide were significantly smaller in terms of both weight and volume than EPI-002 or vehicle control treated animals. This phenomenon implies functional AR remains an integral factor in driving proliferation, despite chronic EPI-002 treatment.

Conclusions:
Taken together these data imply an EPI-specific mechanism of resistance, possibly involving a subset of protein-protein interactions that are altered by EPI-002 treatment yet others are not (specifically those related to growth and survival). As such I intend to look at global gene expression using microarrays to discover what genomic changes have occurred following chronic EPI-002 exposure.

YULIA MERKULOVA

Supervisor: Dr. David Granville
Session: Basic Sciences

"Granzyme B in the Pathogenesis of Chronic Wound Healing: Effects of Granzyme B on Keratinocyte Migration"

AUTHORS:
Yulia Merkulova, Yue Shen, Leigh G. Parkinson, Sheetal A. Raithatha, Hongyan Zhao, David J. Granville

AFFILIATIONS:
[1]Centre for Heart Lung Innovation, St. Paul's Hospital, and Department of Pathology and Laboratory Medicine, University of British Columbia

Background/objectives:
Of the 300 million diabetic individuals worldwide about 15% will develop chronic skin wounds in their lifetime, mainly localized to feet. These wounds often go unnoticed and there are few treatment options available, so many cases result in limb amputation. In fact, every 30 seconds a lower limb is lost to diabetes. Growth factor therapies have been attempted, however, their effects are modest, and these treatments lead to hypercellularity and increased risk of skin malignancies. Thus there is a great need for development of therapeutic strategies to treat non- healing skin wounds. Granzyme B (GzmB) is a serine protease that was, until recently, believed to function exclusively in cytotoxic lymphocyte-mediated apoptosis. However, during excessive or chronic inflammation, GzmB can accumulate in the extracellular milieu, retain its activity, and cleave a number of important extracellular proteins. GzmB is abundant in diseases associated with aging and chronic inflammation such as non-healing skin wounds, rheumatoid arthritis, cancer, and other diseases. Epidermal growth factor receptor (EGFR) is a transmembrane receptor involved in cellular processes such as proliferation, migration, and apoptosis inhibition. EGFR signaling initiated by epidermal growth factor (EGF) binding and subsequent receptor dimerization and autophosphorylation is essential for keratinocyte migration and proliferation during wound healing. In the present study we show that GzmB may contribute to the pathogenesis of chronic wounds by directly inhibiting keratinocyte migration through the EGFR pathway.

Methods:
HaCaT keratinocytes were treated with GzmB (25-200 nM for 6 hours) and the supernatants were immunoblotted for N-terminal extracellular domain of EGFR. Additionally, EGF-induced EGFR phosphorylation was monitored by immunoblotting for phosphorylated EGFR (tyrosine 1068). Immunofluorescent staining of GzmB-treated HaCaT cells followed by confocal microscopy was used to show EGF and EGFR internalization. Scratch assay and Electric Cell-Substrate Impedance Sensing (ECIS) were used to simulate wound healing in vitro and assess keratinocyte migration.

Results:
GzmB prevented EGF-induced EGFR phosphorylation in a dose-dependent manner. Additionally, GzmB reduced the internalization of EGF. Immunofluorescence showed that in GzmB-treated cells, EGF was mainly distributed on the cell membrane while in untreated cells EGF was observed in endosomes and co-localized with EGFR. Lastly, GzmB inhibited the migration of both unstimulated and EGF-stimulated keratinocytes in vitro. While no migration was observed in GzmB-treated/EGF-unstimulated cells over 18 hours, GzmB-treated/EGF-stimulated cells showed a significant delay in migration compared to GzmB-untreated/EGF-stimulated cells, indicating that GzmB impairs keratinocyte migration through an EGFR-dependent mechanism.

Conclusions:
GzmB may contribute to pathogenesis of chronic wounds by disrupting the EGFR signaling pathway, to result in impaired keratinocyte migration. Further characterization of this pathway, and future in vivo studies will provide important proof-of-concept data for developing GzmB- targeted therapies for the treatment chronic skin wounds.

LINNETTE OCARIZA

Supervisor: Dr. Ed Conway
Session: Basic Sciences

"The Protective Effects of Polyphosphate Against Age-related Macular Degeneration Stresses"

AUTHORS:
Linnette M. Ocariza[1,2], Alice M. O'Byrne[1], Stephanie A. Smith[4], James H. Morrissey[4], Jing Z. Cui[3], Joanne A. Matsubara[3], Edward M. Conway[1,2]

AFFILIATIONS:
[1]Centre for Blood Research, Department of Medicine; [2]Department of Pathology and Laboratory Medicine; [3]Department of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver BC; [4]Department of Biochemistry, University of Illinois, Urbana, IL

Background/objectives:
Age-Related Macular Degeneration (AMD) is the leading cause of blindness for people over the age of 50 in the developed world. The pathogenesis of AMD is complex. Evidence suggests that localized oxidative stress and excess complement system activation in the eye are major contributors. These stresses usually lead to the advanced forms of AMD. 'Wet' AMD is characterized by choroidal neovascularization (CNV) and serous leakage into the photoreceptors. 'Dry' AMD is characterized by atrophy of the retinal pigmented epithelial (RPE) cells due to accumulation of drusen – a build-up of cellular by-products, inflammatory proteins, and notably, complement components. Treatments are available only for the 'wet' form, which accounts for ~10% of cases. Polyphosphate (polyP) has recently been discovered as a suppressor of the complement system. PolyP is a ubiquitous, linear, inorganic polymer, the function of which is well-characterized in bacteria, but is only being recently explored in mammals. This project aims to evaluate the mechanisms and potential utility of polyP as a therapeutic agent in AMD. We hypothesized that polyP will confer protection against AMD-related stresses, at least by a complement-dependent mechanism.

Methods:
The eyes of pigment rodents were exposed to laser injuries that induce CNV. PolyP or monophosphate was injected intravitreally immediately after laser injury. Five days later, eyes were excised and flatmounts of choroid/RPE tissues were prepared, immunostained for endothelial cells and deposition of the terminal complement complex C5b-9. Fluorescence was quantified by confocal microscopy and computer analysis. Subsequently, choroidal endothelial cell (CEC) and RPE cell-lines (RF/6A and ARPE-19, respectively) were exposed to oxidative and complement stress, with or without polyP. Nuclear integrity (measured by Hoechst staining) of cultured RPE and CEC in response to H2O2, as well as catalase activity, were examined. Deposition of C5b-9 and release of SC5b-9 were quantified in response to complement activation (using human serum), via flow cytometry and ELISA, respectively.

Results:
Intravitreal polyP reduced the size of laser-induced CNV lesions and deposition of C5b-9 in the choroid of rodents. This led to the investigation of the protective effects of polyP in vitro, including an exploration of oxidative stress effects. These in vitro studies show that treatment of RPE and CEC with polyP dampened C5b-9 deposition and release of SC5b-9 when exposed to complement stress. In addition, polyP conferred protection against against H2O2 -induced nuclear damage. Mechanistically, our data suggest that polyP does not hydrolize H2O2 but up-regulates catalase activity.

Conclusions:
PolyP confers protection against inflammatory and oxidative cellular stresses via complement-dependent and -independent mechanisms. Further evaluation of the mechanistic details is ongoing, especially in regards to the novel finding of the anti-oxidative stress effects of polyP. These results hold promise for the potential of polyP to be a novel therapeutic for AMD, either on its own or in combination with existing therapies.

JACKY LEUNG

Supervisor: Dr. Marianne Sadar
Session: Basic Sciences

"Juglone, a Natural Inhibitor of Pin1, Prevents the Activity of Androgen Receptor and its Splice Variants in Prostate Cancer"

AUTHORS:
Jacky Leung[1,2], Yusuke Imamura[2], and Marianne Sadar[1,2]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Genomic Sciences Centre, British Columbia Cancer Agency

Background/objectives:
The androgen receptor (AR) is a ligand activated transcription factor, serving to maintain the growth and function of the prostate gland in benign physiology and disease. In patients that develop castration-resistant prostate cancer, the AR acquires the ability to sustain signaling in the absence of testicular androgens. Presumably, this is driven by a heightened sensitivity to growth factors or expression of AR splice variants that can restore AR activity. ARv567es and V-7 are AR splice variants that lack the ligand-binding domain, commonly expressed in metastases of patients with castration-resistant prostate cancer. High expression of ARv567es and V-7 is strongly associated with earlier disease progression and shorter survival, as there are currently no conventional therapies that can effectively inhibit their activity. For these reasons, factors that influence sensitivity to ligand-independent pathways or regulate the activity of the AR splice variants are of immediate clinical interest. The regulatory N-terminal domain of the AR contains a number of proline-directed phosphorylation sites, several of which have been proposed to be essential for AR activity. Peptidyl-prolyl isomerase Pin1 is the only enzyme specific for these phosphorylated motifs, and regulates cis/trans isomerization at these unique sites. Juglone (5-hydroxy-1,4-naphthoquinone) is a natural compound produced in roots, nut husks, and leaves of walnut trees (Juglans nigra, Juglans regia, Juglans mandschurica), which have been used traditionally in folk medicine to treat cancer. Juglone is a selective, irreversible inhibitor that covalently binds to the active site of Pin1. The objective of our study was to evaluate the effectiveness of juglone as a potential therapy for prostate cancer **Hypothesis:** We hypothesize that inhibition of Pin1 by juglone can effectively block the activity of AR, as well as constitutively active AR splice variants that lack the ligand binding domain.

Methods:
The effect of Pin1 inhibition by Juglone on AR transcriptional activity was evaluated in human prostate cancer cells (LNCaP) that transiently expressed AR-driven luciferase reporter constructs. The effect on AR transactivation was performed in LNCaP cells stimulated with cAMP activator forskolin or cytokine interleukin-6. The effect on the basal transcriptional activity of AR splice variants was tested in an AR-negative cell line (PC-3), using an AR-driven luciferase reporter. Cell proliferation in LNCaP cells that express full-length AR and LNCaP95 cells that express both full-length and AR splice variants was assessed by BrdU labeling and measured by an ELISA method.

Results:
Our results show that juglone effectively inhibited AR transcriptional activity and decreased phosphorylation on AR at a proline-directed site, serine81. Interestingly, juglone selectively blocked transactivation of the AR N-terminal domain in the presence of interleukin-6, but not forskolin. Furthermore, juglone abrogated the constitutive activity of AR splice variants, and inhibited proliferation of androgen-sensitive LNCaP cells and androgen-independent LNCaP95 cells.

Conclusions:
Currently there are no effective treatments for patients with castration-resistant prostate cancer, particularly for patients with detectable levels of AR splice variants. We provide evidence supporting that Pin1 is an important regulator of AR activity, and show that by inhibiting Pin1 with juglone we can block the activity of AR and its splice variants. For these reasons, Pin1 is an alluring therapeutic target. Our findings suggest that the development of inhibitors targeting context-specific mechanisms is an encouraging strategy for effective treatment of castration-resistant prostate cancer.

SIGRID ALVAREZ

Supervisor: Dr. Bruce Verchere
Session: Basic Sciences

"Islet Expression of Interleukin-35 Protects from Autoimmune Diabetes and Islet Transplant Rejection"

AUTHORS:
Sigrid Alvarez[1], Loraine Bischoff[1], Derek L. Dai[2], Galina Soukhatcheva[2], Paul C. Orban[2], C. Bruce Verchere[1,2]

AFFILIATIONS:
Child & Family Research Institute; [1]Department of Pathology and Laboratory Medicine; [2]Department of Surgery, University of British Columbia

Background/objectives:
Interleukin-35 (IL-35) has recently been identified as a cytokine expressed by T regulatory cells and B cells with the ability to suppress conventional T cell proliferation and effector functions. Transgenic beta cell expression of IL-35 protects NOD mice from autoimmune diabetes associated with decreased insulitis and a reduction in CD4+ and CD8+ T cells numbers. In this study, we sought to determine whether a gene therapy approach could be used to express IL-35 for prevention of autoimmune diabetes and protection against islet allograft rejection.

Methods:
To express IL-35 specifically in beta cells, we generated an adeno-associated virus serotype 6 expressing IL-35 downstream of the rat insulin promoter (AAV6-RIP-IL-35). Non obese diabetic (NOD) females received either AAV6-RIP-IL-35 or AAV6-RIP-empty by injection via the pancreatic duct. Diabetes development was monitored by weekly blood glucose measurements. For allogeneic transplantion, streptozotocin-treated Bl/6 female mice received Balb/c islets transduced with AAV6-RIP-IL-35 ex vivo. To test for graft tolerance in recipients of IL-35-expression islet allografts, the graft-bearing kidney was removed and untreated allogeneic islets were transplanted into the contralateral kidney.

Results:
To express IL-35 specifically in beta cells, we generated an adeno-associated virus serotype 6 expressing IL-35 downstream of the rat insulin promoter (AAV6-RIP-IL-35). Non obese diabetic (NOD) females received either AAV6-RIP-IL-35 or AAV6-RIP-empty by injection via the pancreatic duct. Diabetes development was monitored by weekly blood glucose measurements. For allogeneic transplantion, streptozotocin-treated Bl/6 female mice received Balb/c islets transduced with AAV6-RIP-IL-35 ex vivo. To test for graft tolerance in recipients of IL-35-expression islet allografts, the graft-bearing kidney was removed and untreated allogeneic islets were transplanted into the contralateral kidney.

Conclusions:
Beta cell expression of IL-35 early in the course of disease protects NOD mice from autoimmune diabetes development. Additionally, IL-35 is able to protect against islet allograft rejection and may induce donor-specific tolerance. These data demonstrate the potential of IL-35 as a therapy for autoimmune diabetes and islet transplantation.

MATTHEW BUDD

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"An Examination of Mitochondrial DNA Abnormalities in the Blood of HIV-exposed Uninfected Children with Autism Spectrum Disorder and in a Mouse Model of Autistic-like Behaviour"

AUTHORS:
Matthew A Budd[1,2], Jason Brophy[3], Jennifer Bowes[3], Lindy Samson[3], Christopher Shaw[4], Sneha Sheth[4], Hélène C.F. Côté[1,2] and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Centre for Blood Research, University of British Columbia; [3]Department of Pediatrics, Children's Hospital of Eastern Ontario, University of Ottawa; [4]Department of Ophthalmology and Visual Sciences, University of British Columbia

Background/objectives:
Autism spectrum disorder (ASD) is a range of neurodevelopmental diseases resulting in repetitive behaviour and impaired social ability. In 2010, the CDC estimated the prevalence of ASD at 1.47% among U.S. children, up from 0.66% in 2002. Pediatricians at the Children's Hospital of Eastern Ontario noticed a high rate of ASD among their HIV-exposed uninfected (HEU) patients. A review of 296 HEU children enrolled in the Children & Women AntiRetrovirals & Mechanisms of Aging (CARMA) cohort identified 16 (5.41%) children with a diagnosis of ASD and a further 3 with strong suspicion of ASD, in total a fourfold increase over the normal rate. Many HEU children in the cohort are under 3 years, a typical age for ASD diagnosis, so this number may rise. Only 2 cases of ASD were identified in the cohort's 141 HIV+ children (1.42%). Prior studies have suggested associations between ASD and mitochondrial dysfunction, as well as maternal infections in pregnancy. Abnormalities in mitochondrial DNA (mtDNA) quantity and quality, and increases in oxidative stress, have also been observed in children whose HIV+ mothers received antiretroviral (ARV) therapy during pregnancy. Our objective is to determine whether HEU children with ASD have greater blood mtDNA alterations than both HEU children without ASD and HIV-unexposed uninfected (HUU) children. In parallel, brain and blood tissue from mice displaying ASD-like behaviour will also be investigated.

Methods:
Blood samples (collected between 7 months and 16 years of age) from HEU children (n=19: 14 males, 5 females) with diagnosed and suspected cases of ASD will be studied. We will match these children 2:1 by sex, age, and ethnicity to HEU children without ASD (n=38), and by sex and age to anonymous HUU controls (n=38) for whom no other information is available. As some ASD children have multiple blood specimens available, we will also match them for the purposes of longitudinal comparison to children who have specimens taken at similar ages. Leukocyte mtDNA/nuclear DNA ratio and mtDNA apparent oxidative damage will be measured using qPCR-based assays. MtDNA mutation burden will be measured via a next-generation sequencing method that allows quantification of rare somatic mutations that are below the limit of detection of currently available techniques. Associations with maternal factors (e.g. substance use in pregnancy, maternal co-infections, and type and duration of maternal ARV in pregnancy) will also be explored.

In the CD-1 mouse model, mtDNA from blood and brain tissue will be analyzed in mice injected neonatally with a toxin that induces behavioural phenotypes consistent with those of ASD (n=9), and their results will be compared with those of control mice (n=7).

Significance:
As many as three million HEU children are born each year; a large percentage of them exposed to ARVs in utero. The apparent high prevalence of ASD in HEU children is a concern given that both HIV and ARVs are shown to affect mtDNA, and possibly mitochondrial function. If we detect an association between ASD and mtDNA alterations in HEUs, maternal factors, or in utero exposures, this could open important research avenues.

SAMANTHA BURUGU

Supervisor: Dr. Torsten Nielsen
Session: Basic Sciences

"The Prognostic Value of Tumor-infiltrating Lymphocytes in Leiomyosarcoma"

AUTHORS:
Samantha Burugu[1], Matt van de Rijn[2] and Torsten Nielsen[1]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Department of Pathology, Stanford University Medical Center

Background/objectives:
With the emergence of successful cancer immunotherapy agents for some tumor types, the cancer research field is increasingly studying the tumor immunology. As an example, ipilimumab, an antibody cancer drug targeting an inhibitory protein located on T lymphocytes cellular membrane called cytotoxic T-lymphocyte associated protein 4, has resulted in impressive clinical results in patients with metastatic melanoma. The presence of specific lymphocytes infiltrating tumors such as cytotoxic T cells (CD8+) and regulatory T cells (FOXP3+) has a prognostic value and has been associated with good or bad prognoses in different tumor types. In the case of leiomyosarcoma, a smooth muscle cancer and one of the most common types of sarcoma, the prognostic value of immune markers has been studied only by looking at tumor-associated macrophages biomarkers. Leiomyosarcoma is an aggressive cancer with a 64% 5-year survival rate. Our objective was to survey the presence of T lymphocytes such as cytotoxic T cells, helper T cells, regulatory T cells and markers of activated T cells identified by CD8, CD4, FOXP3 and PD-1 positivity, respectively and to assess their potential prognostic value in leiomyosarcoma primary tissues.

Methods:
126 leimyosarcoma primary tissues represented on a tissue microarray were single stained with CD4, CD8, FOXP3 and PD-1 antibodies by immunohistochemistry using Ventana automated instrument. The positive cells were counted . All descriptive and survival analyses were conducted using SPSS software (PAWS Statistics version 18).

Results:
Presence of CD4+, CD8+, FOXP3+ and PD-1+ cells was observed in 47.6%, 79%, 49.5% and 8.5% of cases, respectively. Among the markers tested, significant positive correlations were observed between the presence of CD8+ cells and tumor size (r=0.220, P=0.035) and between the presence of FOXP3+ cells and age (r=0.203, P=0.038). In survival analyses, there were negative correlations between the presence of immune markers (CD4+, CD8+, and FOXP3+) and disease-specific survival but these did not reach statistical significance.

Conclusions:
Overall, to our knowledge, this is the first study assessing the presence and prognostic value of CD4+, CD8+, FOXP3+ and PD-1+ cells in leiomyosarcoma. Survival analyses in PD-1+ cases could not be computed due to their low frequency. The negative trend observed between the presence of T lymphocytes (CD4+, CD8+, FOXP3+) and disease-specific survival did not reach statistical significance. Nevertheless, this trend is in agreement with previous studies showing that the presence of tumor-associated macrophages in leiomyosarcoma is associated with a poor clinical outcome. Future directions will involve analyzing prognostic value of additional immune biomarkers in different types of sarcomas.

EMILY BUTTON

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"High Density Lipoproteins Reduce Inflammation in Human Brain Microvascular Endothelial Cells"

AUTHORS:
Emily Button[1], Jerome Robert[1], Sophie Stukas[1], Cheryl Wellington[1]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, Djavad Mowafaghian Centre for Brain Health, University of British Columbia

Background/objectives:
Alzheimer's disease (AD) is the most common cause of senile dementia. Vascular changes including reduced cerebral blood flow, cerebrovascular density, and blood-brain barrier integrity have been observed in AD and represent an understudied aspect of AD pathogenesis.

High density lipoproteins (HDL) have gained acceptance as a beneficial compound for peripheral vascular health. However, it has yet to be shown if HDL acts similarly in the cerebrovasculature. This work aimed to investigate whether the anti-inflammatory functions of HDL can be observed in cultured human brain microvascular endothelial cells.

Methods:
Plasma HDL was isolated from healthy human volunteers by density gradient ultracentrifugation and purity was confirmed by denaturing SDS-PAGE. HDL induced nitric oxide (NO) production was assessed in human brain microvascular endothelial cells (hBMECs) using a diaminofluorsecin-2 diacetate (DAF-2 DA) assay. DAF-2 DA reacts with NO to produce a fluorescent derivative which can be measured and quantified. The ability of HDL to suppress tumour necrosis factor alpha (TNFa) stimulated inflammation was investigated through the measurement of interleukin-6 and vascular cell adhesion molecule expression levels by ELISA and western blotting respectively.

Results:
HDL isolated from healthy human donors significantly increased nitric oxide production in hBMECs. HDL significantly suppressed TNFa induced inflammation as seen by elevation in IL-6 and VCAM expression.

Conclusions:
The anti-inflammatory properties of HDL in the vasculature are becoming well established through work in the cardiovascular disease field. HDL modifying therapeutics may be a promising strategy in maintaining cerebrovascular health as shown by this work, which would be of benefit to those with Alzheimer's disease due to the emerging vascular component of disease pathogenesis.

JACK CALDER

Supervisor: Dr. Will Lockwood
Session: Clinical Sciences

"Identification and Characterization of the Molecular Target of a Novel Small Molecule Inhibitor of Lung Cancer"

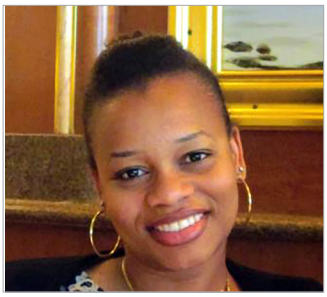
AUTHORS:
Jack Calder, Will Lockwood

AFFILIATIONS:
[1]BC Cancer Research Center

Background/objectives:
Lung Cancer (LC) is the leading cause of cancer death worldwide, mainly due to the lack of effective drugs available. Difficulty in target-based drug discovery has led to a renewed interest in phenotypic/function based screening for the identification of novel small-molecule inhibitors. Through a screen of 189, 290 small molecules, we have identified a compound known as lung cancer screen 3 (LCS3) that is structurally different from most known drugs and found to be a broad-spectrum inhibitor of cancer cells while not being toxic to normal cells. Early indications from affinity approaches using LCS3-linked beads and gene knockout cell lines indicate that the primary target of LCS3 is DDX3X, an ATP dependant RNA helicase.

Methods:
Lung Cancer (LC) is the leading cause of cancer death worldwide, mainly due to the lack of effective drugs available. Difficulty in target-based drug discovery has led to a renewed interest in phenotypic/function based screening for the identification of novel small-molecule inhibitors. Through a screen of 189, 290 small molecules, we have identified a compound known as lung cancer screen 3 (LCS3) that is structurally different from most known drugs and found to be a broad-spectrum inhibitor of cancer cells while not being toxic to normal cells. Early indications from affinity approaches using LCS3-linked beads and gene knockout cell lines indicate that the primary target of LCS3 is DDX3X, an ATP dependant RNA helicase.

Results/ Conclusions:
Recent data shows that knockdown of DDX3X selectively kills cancer cell lines but not normal or resistant cell lines, providing additional evidence for DDX3X as the target of LCS3-mediated killing. However, additional experiments are required and ongoing to confirm this association. Together, this work will lead to a better understanding of the underlying mechanisms that lead to lung tumorigenesis and the mechanisms of action of LCS3.



ROLINDA CARTER

Supervisor: Dr. Ed Pryzdial
Session: Basic Sciences

"Novel Role for Direct Xa Anticoagulants in Fibrinolysis"

AUTHORS:
Rolinda R.L. Carter[2,3], Kimberley Talbot[1,2,3], Tyler W Smith[4], Agnes Y.Y. Lee[3,4] and Ed L.G. Pryzdial[1,2,3]

AFFILIATIONS:
[1]Canadian Blood Services, Centre for Innovation; [2]Department of Pathology and Laboratory Medicine; [3]Centre for Blood Research, University of British Columbia, [4]Department of Medicine, Division of Hematology, University of British Columbia

Background/objectives:
The balance between the formation and dissolution of a blood clot is vital for the prevention of hemorrhage and clot-induced heart attacks or stroke. Thus, the presence of an aberrant clot blocking the flow of blood is a major contributor to cardiovascular disease, the leading cause of death worldwide. To dissolve these clots, the key physiological clot-busting enzyme, tissue plasminogen activator (tPA) has been developed as a drug. It works by converting plasminogen into its active form plasmin, the enzyme that cleaves and solubilizes the clot. tPA, however, is not a perfect drug. Due to its rapid clearance rate, it is given at a high dose to be effective and this may result in cerebral hemorrhage. As an alternative target for development, we have found that plasmin-cleaved clotting factor Xa (FXa) enhances clot dissolution by accelerating the activity of tPA at low physiological concentrations. This novel function of FXa can be further enhanced or lost by modifying its active site and thus changing its fragmentation profile. The newest generation of anticoagulant drugs, rivaroxaban (riva) and apixaban (apix), inhibit FXa by binding the active site. Their consequent effect on the function of FXa as a clot dissolving agent has not been considered. Here we address the hypothesis that riva and apix will alter the modulatory cleavage sites of FXa and affect its subsequent participation in clot-dissolution.

Methods:
Thrombin-initiated clot formation and dissolution were monitored by Rayleigh light scattering in normal plasma, FX-deficient plasma, and FVIII-deficient plasma. Thrombin is the enzyme that converts soluble fibrinogen into its polymerized form, fibrin, the molecular mesh of clot. To bypass any experimental variables due to in situ thrombin generation, an excess of thrombin was desirable. This is because the addition of Xa and riva or apix in these experiments may increase or decrease thrombin generation, respectively. The fragmentation profile of FXa was evaluated by immuno-blot to compare normal plasma, plasma from a patient on prophylactic riva, and normal pooled plasma containing riva.

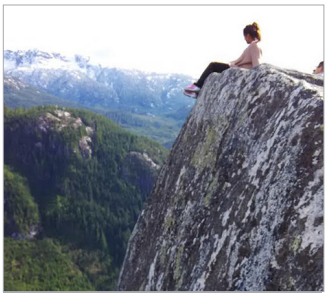
Results:
In plasma, riva and apix enhanced clot dissolution in normal plasma. Effort to determine the basis for this enhancement revealed by immuno-blot analysis that riva stabilizes FXa in a form previously shown to enhance the dissolution of plasma clots. This observation was made in riva patient plasma and normal plasma spiked with riva. Using FX-deficient plasma, it was confirmed that the enhancement of riva is FX-dependent. Riva also enhanced plasma clot lysis in FVIII-deficient plasma, which minimizes in situ thrombin generation.

Conclusions:
This study suggests a previously unknown dual role for riva and apix as an inhibitor of clot formation and an enhancer of clot dissolution. Understanding how these new generation anticoagulants affect the mechanisms that control clot formation and clot dissolution may help to explain why life-threatening bleeding may occur in some patients. It may furthermore open avenues to treating post-thrombotic syndrome, a serious healthcare burden with possible links to clot persistence.

TANYA DE SILVA

Supervisor: Dr. Will Lockwood
Session: Clinical Sciences

"The Role of SHPRH in Never Smoker Lung Adenocarcinoma"



AUTHORS:
Will Lockwood, **Tanya de Silva**

AFFILIATIONS:
Department of Pathology and Laboratory Medicine, University of British Columbia; BC Cancer Research Centre

Background/objectives:
Lung cancer is the leading cause of cancer related death within Canada and worldwide. Although a significant fraction of lung cancers are in smokers, many arise in never smokers, which globally represent over 300,000 deaths per year. Lung cancer in never smokers is particularly deadly as it is usually identified at an advanced stage where there are no curable treatments available. Thus, there is an urgent need to identify early detection markers and improved therapeutic regimens for this subset of lung cancer patients. Despite mounting evidence of familial susceptibility and genetic linkage to lung cancer, the specific genes responsible have yet to be determined. Identifying such genes may hold the link to determining the causes of lung cancer susceptibility and reveal an opportunity for early diagnosis and targeted therapy. Our group has examined recurrent patterns of genetic variation in never smoker lung tumors in order to identify those that may predispose people who have limited exposure to tobacco (never smokers) to lung cancer.

Methods:
Through sequencing of sporadic (i.e. non familial cases) lung cancers, we have identified the disruption of a gene called SHPRH within a region linked to familial lung cancer. Given that SHPRH has already been implicated as a potential tumor-suppressor and its location within the lung cancer susceptibility locus, we hypothesize that SHPRH inactivation may offer a proliferative and/or survival advantage to lung cells during tumor formation. Although this gene is known to play a critical role in regulating DNA repair and genomic instability, its functional role in cancer progression remains to be elucidated. Our preliminary results establish a link between SHPRH expression and proliferation rate of 'normal' lung lines, suggesting a potential role of SHPRH in lung cancer development. Currently, further gain/loss of function studies are underway comparing differences in SHPRH expression or disruption of its interplay within its protein complex and how this may impact transformation and chromosomal instability. Next, we will induce expression of SHPRH in lung cancer cells that have lost SHPRH due to gene disruption to determine if this reverses the malignancy of these cells.

Conclusions:
This work will investigate the relationship between SHPRH variability and lung cancer susceptibility on a number of different fronts, including comprehensive studies integrating genomic profiles of pre-invasive and invasive human lung tumors with gain/loss of function approaches and mouse models of lung cancer to determine the effect on lung cancer initiation and development. This study will delineate the key functional roles of SHPRH and determine whether disruption of this gene is involved in lung cancer predisposition with the ultimate goal of designing new avenues for early diagnostic and therapeutic intervention for never smoker lung cancer patients.

HAOYU DENG

Supervisor: Dr. Honglin Luo
Session: Basic Sciences

"Enhanced Enteroviral Infectivity via Viral Protease-mediated Cleavage of Grb2 Associated Binder1"

AUTHORS:
Haoyu Deng[1], Gabriel Fung[1], Junyan Shi[1], Chen Wang[1], Jun Hou[1], Jingchun Zhang[1], Zheng-Gen Jin[2,*], Honglin Luo[1,*]

AFFILIATIONS:
[1]Centre for Heart Lung Innovation, St. Paul's Hospital and Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver; [2]Aab Cardiovascular Research Institute and Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Background/objectives:
As a major pathogen causing viral myocarditis, coxsackievirus B3 (CVB3) utilizes virus-encoded viral protease 2Apro and 3Cpro to cleave specific host proteins crucial for maintenance of cell survival and signalling transduction in an attempt to optimize the conditions for facilitating viral replication. Grb2 associated binder1 (Gab1) is a first-recognized docking protein in charge of intracellular signaling assembly and transduction, which plays an important role in maintaining cell survival. Recent discovery of cleavage of Gab1 induced by CVB3 infection emphasizes a potentially important role of Gab1 in regulating viral infection.

Methods:
HeLa cell lysates were harvested after CVB3 infection and then processed by western blot for determining the protein levels of Gab1. Subsequently, potential cleavage sites on Gab1 mediated by viral proteases were predicted using NetPicoRNA 1.0 server and confirmed by site-directed mutagenesis techniques. To determine the functional role of Gab1 in modulating viral replication and ERK pathway, Gab1 was either knocked down or overexpressed. The protein levels of VP1 and phosphorylation of ERK1/2 (pERK1/2) were determined by Western Blot. Simultaneously, the same experiments were performed to examine the effects of Gab1 cleavage products on regulating CVB3 life cycle and host ERK pathway. Finally, immunostaining were performed to determine the localizations of cleaved Gab1.

Results:
Our studies demonstrated that 7hr CVB3 infection resulted in Gab1 cleavage and the formation of ~75KDa and ~40KDa fragments. We showed that inhibition of caspase activation using z-VAD-fmk, failed to block the formation of viral-induced ~75KDa and ~40KDa cleavage fragments. Furthermore, we identified that Gab1-G175 and Gab1-G436 were targeted by viral protease 2Apro as two cleavage sites during CVB3 infection. Upon CVB3 infection in Gab1 knockdown cells, our data demonstrated a significant attenuation of viral protein expression. Moreover, we demonstrated that Gab1-N1-174 translocated to the plasma membrane via its PH domain upon CVB3 infection. Finally, we provided evidence that Gab1-N1-174 facilitated CVB3 replication via further enhancing the ERK signaling pathway.

Conclusions:
Our study suggests a novel mechanism by which CVB3 targets host Gab1 to generate a Gab1-N1-174 fragment that enhances viral infectivity via upregulation of the ERK pathway.

SARAH FERNANDO

Supervisor: Dr. Neil Cashman
Session: Basic Sciences

"Extracellular Vesicles from Amyotrophic Lateral Sclerosis Tissue have Misfolded SOD1 Cargo and Are Implicated in Propagation of Protein Misfolding"

AUTHORS:
Sarah M. Fernando[1], Judith M. Silverman[2], Leslie I. Grad[2], Neil R. Cashman[1,2]

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

Background/objectives:
Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease affecting approximately 450,000 people worldwide. Motor neurons in ALS patients progressively degenerate over time causing paralysis of voluntary muscles. In these patients, pathology is observed to spread spatiotemporally through the neuroaxis from one contiguous area to the next, reminiscent of a prion-like mechanism for disease propagation. Mutations in Cu/Zn superoxide dismutase (SOD1) are linked to inherited cases of ALS, and misfolded SOD1 (misSOD1) protein is found in ALS tissues. We have previously shown that wild-type human SOD1 in cultured cells can be induced to misfold via contact with mutant misSOD1, and that cell-to-cell transmission of misfolding can occur between cells via the uptake of exosomes. However, the mechanisms by which misSOD1 is propagated in vivo remain ambiguous. We hypothesize that extracellular vesicles (EVs) from ALS neuronal tissues bear misSOD1 and act as a mode of transportation for propagation of misSOD1 seen in ALS.

Methods:
We isolated EVs from frozen neuronal tissues of ALS mouse models and human ALS patients by serial centrifugation and purification using density gradients. Resultant vesicle populations were examined via transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and immunoblotting.

Results:
We found that vesicle populations pelleted at 10,000g and 100,000g and localized to the ~30% sucrose density fraction displayed characteristic circular cup-shaped morphology under TEM. Both EV populations were positive for neuronal EV-specific markers such as prion protein, flotillin1, and wild-type SOD1. Markers of intracellular contamination were largely absent in the isolations, confirming cellular integrity during the isolation protocol. Notably, we observed highly polydisperse vesicle diameters under TEM, which was confirmed using NTA technology. Next, immunoprecipitation using antibodies specific for misSOD1 showed the enrichment of misSOD1 on ALS tissue-derived EVs compared to non-ALS controls. We applied the secreted EV-containing fraction from mutant-SOD1 expressing cells onto wild-type cells in culture and observed that it induced misfolding of SOD1 in those cells, a phenomenon which was abolished by heat-denaturing the EV-containing fraction at 96°C prior to treatment of cells. Finally, our preliminary results suggest that the application of ALS mouse tissue-derived EVs onto HEK cells in culture also causes misfolding of wild-type SOD1 in those cells.

Conclusions:
In summary, we have successfully isolated EV populations from ALS patient and murine neuronal tissues, and shown these vesicles to specifically carry misfolded SOD1. Our results suggest that EVs bearing misfolded SOD1 are competent to induce misfolding of wild-type SOD1, implicating EV dissemination in the propagation of SOD1 misfolding seen in ALS.

ABSTRACT #33

Graduate Student | Poster Session

CHRISTA KLEIN-BOSGOED

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

"Protein Synthesis in Blood Platelets: How Platelet mRNA is Affected by Pathogen Inactivation Treatment"

AUTHORS:
Christa Klein-Bosgoed[1,2], Peter Schubert[1,2,3] and Dana V. Devine[1,2,3]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine; [2]Centre for Blood Research, University of British Columbia, and [3]Centre of Innovation, Canadian Blood Services

Background/objectives:
Human blood platelets are essential blood components that are responsible for maintaining hemostasis. Canadian Blood Services produces almost 115,000 platelet products annually for transfusion. These platelet concentrates (PCs) are used to treat patients, e.g., with a low platelet count or patients undergoing chemotherapy. To increase the safety of blood products, new techniques are being developed to inactivate potential pathogens in PCs. These pathogen inactivation (PI) techniques use UV illumination with or without a photosensitizer to irreversible damage viral and bacterial RNA and DNA. Even though platelets lack a nucleus and have no innate DNA transcription, it has been demonstrated that platelets have the ability to synthesize proteins using the RNA pre-deposited by megakaryocytes. The role of this protein synthesis in platelet, however, still remains unclear. It has also yet to be determined if PI affects platelet protein synthesis and platelet function. In this study we determine to what extend platelet mRNA is affected by PI. We hypothesize that application of PI, which is designed to target viral and bacterial RNA and DNA, has a negative impact on platelet mRNA.

Methods:
Apheresis PCs were collected from healthy volunteer donors according to standard procedures. In a pool and split design, one arm was PI treated while the other arm served as an untreated control. Samples were taken one hour or one day after illumination. RNA was extracted using Trizol® (Invitrogen, Burlington ON) according to the manufacturers' instruction. The mRNA levels of the glycoproteins (GP)IIIa, GPIIb (subunits of the fibrinogen receptor) and GPIb (subunit of the vWF receptor) were determined using quantitative polymerase chain reaction (qPCR). The alpha-granule proteins PF4, SPARC and TSP, previously demonstrated by our lab to change their protein expression after PI treatment, were investigated as well. GAPDH was used as a target to evaluate the effect of PI on a housekeeping gene. The mRNA levels were normalized to the untreated control sample.

Results:
For all selected targets, with the exception of GPIb, the amount of mRNA was significantly reduced one hour after UV illumination and further reduced after 24 hours. Interestingly, the PI treatment affected the selected mRNAs differently. For GAPDH and PF4, around 70 % of the mRNA was still detectable one hour after UV illumination, making them less susceptible for mRNA degradation due to PI compared to GPIIIa and TSP where less than 15 % was detected after PI treatment. This observation can in part be explained by a correlation of number of guanine bases in the mRNA transcript.

Conclusions:
Even though in this study only a few targets were analyzed, the results suggest that all mRNA present in human blood platelets are susceptible to PI, including mRNA of housekeeping genes. The overall impact of PI on platelet mRNA is dependent on characteristics of the mRNA transcript itself and possibly on external factors, like the UV dose applied, which are currently under investigation.

ROBERT KRIDEL

Supervisor: Dr. Randy Gascoyne
Session: Clinical Sciences

"Cell of Origin Assignment in Transformed Follicular Lymphoma"

AUTHORS:
Robert Kridel[1]*, Anja Mottok[1]*, Pedro Farinha[1], Susana Ben-Neriah[1], King Tan[1], Daisuke Ennishi[1], Fong Chun Chan[1], Merrill Boyle[1], Adele Telenius[1], Barbara Meissner[1], Laurie H. Sehn[1], Joseph M. Connors[1], Marco A. Marra[2], Sohrab P. Shah[3], Christian Steidl[1], David W. Scott[1], Randy D. Gascoyne[1]
**equal contribution*

AFFILIATIONS:
[1]Centre for Lymphoid Cancer; [2]Genome Sciences Centre; [3]Molecular Oncology, BC Cancer Agency

Background/objectives:
In follicular lymphoma (FL) the median overall survival time for newly diagnosed patients is currently well beyond 10 y. Nonetheless, in 20-30% of patients, within 10 y the disease transforms into an aggressive lymphoma subtype, an event associated with increased morbidity, need for treatment and risk of lymphoma-related death. Despite an increasingly refined understanding of the mutational landscape of transformed follicular lymphoma (TFL), the biological correlates underpinning poor outcome for TFL patients are imperfectly understood. In a prior study assessing the gene expression profile of TFL, all classifiable cases (n=18) were of germinal centre B-cell (GCB) phenotype and none were of the activated B-cell (ABC) phenotype, although 3 cases out of an independent cohort of 35 (9%) were assigned to the non-GCB phenotype using immunohistochemistry (Davies et al, BJH, 2007). As subtype-specific efficacy of novel agents is actively pursued in *de novo* diffuse large B-cell lymphoma (DLBCL), it is relevant to ask whether TFL can be similarly divided into distinct transcriptional phenotypes.

Methods:
Out of a cohort of 148 formalin-fixed and paraffin-embedded TFL samples, 112 (76%) had a morphology akin to DLBCL, as opposed to unclassifiable B cell lymphoma (BCLU) or composite histologies. We applied the Lymph2Cx assay, a digital gene expression (NanoString)-based test, to RNA extracted from these 112 samples, as previously described (Scott et al, Blood, 2014). A tissue microarray was constructed and chromosomal rearrangements of BCL2, BCL6 and MYC were assessed by fluorescence in situ hybridization (FISH) breakapart assays.

Results:
The Lymph2Cx assay provided sufficient digital gene expression counts to allow for class assignment in 109 out of the 112 cases (97%). Of these 109 samples, 87 (80%) were assigned to the GCB subtype, 17 (16%) to the ABC subtype and 5 (4%) were unclassified. BCL2 translocations were found in 65 out of 72 cases (90%) that were interpretable by FISH and that had a GCB phenotype, but only in 5 out of 14 cases (36%) with an ABC phenotype. This difference was statistically significant ($p<0.001$). The prevalence of BCL6 and MYC translocations did not significantly differ between the subtypes.

Conclusions:
Our data show that a majority of TFL cases with DLBCL morphology (80%) are of the GCB subtype when assessed using the Lymph2Cx assay. However, a significant minority (16%) are of the ABC subtype and characterized by a low prevalence of BCL2 translocations. Future studies are needed to assess whether these subtypes differ in outcome and molecular ontogeny, which would potentially make them amenable to distinct therapeutic targeting.

GUNJAN KUMAR

Supervisor: Dr. Mads Daugaard
Session: Basic Sciences

"Functional Cloning of Cisplatin Resistance Pathways in Bladder Cancer"

AUTHORS:
Gunjan Kumar[1], Mads Daugaard[1,2], Peter Black[1,2]

AFFILIATIONS:
Vancouver Prostate Centre;
[1]Department of Pathology and Laboratory Medicine;
[2]Department of Urology, University of British Columbia

Background/objectives:
Urothelial carcinoma (bladder cancer) is the 4th most common cancer in men and 9th most common cancer in women. The 5 year disease-free survival rate for this cancer is 80%. This number drops to about 6% in the metastatic disease (approximately 10% of cases). Accounting for almost $3.7 billion in national healthcare costs, bladder cancer is the 5th most expensive disease to treat. The most common form of bladder cancer is transitional cell carcinoma (TCC), making up about 90% of the cases. A commonly employed treatment for bladder cancer is a transurethral resection of the bladder tumour followed by an adjuvant chemotherapy usually with a combination of cisplatin and gemcitabine. Unfortunately however, TCC cells develop a multidrug resistance to the treatment and the surviving cells have the ability to proliferate and repopulate the tumour. The recurrent tumour is highly resistant to the chemotherapy, which poses a significant clinical challenge in the treatment of bladder cancer. While work in the field is ongoing, the mechanism of drug resistance is still largely unclear which means that therapy options are almost non-existent. Hypothesis: Complementary DNA (cDNA) built from a primary resistant tumour source can be used to identify molecular components able to confer treatment resistance in sensitive bladder cancer cells.

Objectives and Rationale: The main objective of this study is to identify molecular RNA and protein components that confer cisplatin treatment resistance in bladder cancer. Identified components may then be regarded as bona fide targets for therapeutic intervention strategies to break resistance.

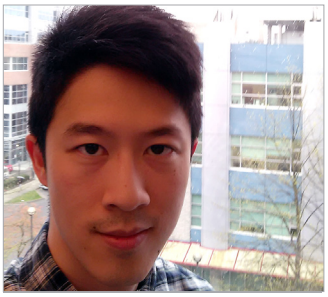
Methods:
Cisplatin resistant cell lines were generated in-vivo in Nude mice using orthotopic bladder tumours. Mouse tumours were treated with cisplatin at regular intervals until a resistance was achieved. These tumours were then excised and broken down into single cell cultures. Cisplatin dose assays were performed on these resistant cells to determine their IC50 compared to parental (naïve) cells. A 72 hour treatment with 25uM of cisplatin after a 24 hour serum starvation is the optimal condition to eliminate all parental cells.

Results and Conclusion:
The resistant cell lines generated in-vivo can be used as positive controls for a resistant phenotype to be compared to the resistant that will be conferred after transfection with the cDNA library. The resistance phenotype is further confirmed by the visualization of the cellular morphology upon treatment with cisplatin. Theoretically, cDNA transfected cells which have acquired resistance should have similar IC50 to the resistant cells generated in-vitro and in-vivo. Since cisplatin is a DNA-damaging agent which is most effective during the S phase of the cell cycle, subjecting cells to growth arrest followed by release and treatment will ensure that a maximal number of cells are exposed to cisplatin during the S-phase. This will be useful in the elimination of background when the transfection is carried out and the acquired resistance is analyzed with a high degree of accuracy. The next step of this project would be to extract mRNA from chemo-resistant patient samples and construct a cDNA library from these extracted mRNA which would then be introduced into parental cells to determine changes in resistance. Finally, with this project, identifying genes which confer resistance to the bladder cancer cells would yield insight into the changes which are necessary for resistance to develop. This would thus pave the way for targeted therapeutic alternatives which work to reverse this resistance, giving the patient a better disease prognosis.

BRYAN LIN

Supervisor: Dr. Ed Pryzdial
Session: Basic Sciences

"Coagulation Triggered by Virus and Host Constituents Integral to the Virus Surface"



AUTHORS:
Bryan Lin, Michael Sutherland and Ed Pryzdial

AFFILIATIONS:
Department of Pathology and Laboratory Medicine, University of British Columbia; Canadian Blood Services Research and Development, Centre for Blood Research, University of British Columbia

Background/objectives:
Many prothrombotic and/or proatherogenic viruses have an outer envelope structure containing host-derived membrane lipids and proteins, as well as virus-encoded proteins. Our lab uses herpes simplex virus type 1 (HSV1) as an enveloped virus model since it is well-characterized and relatively safe to propagate and purify. We have shown that the HSV1 envelope acquires tissue factor (TF) from the host, a protein critical in the initiation of physiological blood coagulation. Normally, functional TF is available to circulating plasma proteins only upon vascular damage. Viral TF accelerates the enzyme factor VIIa, where the TF-factor VIIa complex activates factor X (FX) to FXa, leading to the formation of a clot. Without TF, virus infectivity is compromised in vitro and in vivo, as evidenced by our lab. The virus-encoded glycoprotein C (gC), a complement inhibitor and cell attachment protein, was shown by our lab to enhance factor VIIa activity and bind to FX, on the virus surface and in solution using soluble recombinant proteins lacking transmembrane domains. These data suggest gC may have evolved to affect host-encoded TF function. How viral gC augments host-derived TF-mediated FX activation and hence participate in vascular disease is unknown. To address the hypothesis that HSV1-encoded gC interacts with TF and FX on the virus surface to enhance factor VIIa-dependent FX activation, I will be pursuing the following aims: Aim 1; to demonstrate the effect of HSV1 gC on TF-dependent FX activation on TF+/- HSV1. Aim 2; to dissect the role of gC in TF-factor VIIa complex assembly and/or function.

Methods:
HSV1 (NS strain) and a gC deficient sister strain (ns-1) have been propagated in a human melanoma cell line A7/TF, which can inducibly express TF to obtain gC+/TF-, gC+/TF+, gC-/TF+ or gC-/TF-viruses. To follow the activation of factor X, a factor Xa-selective chromogenic substrate was used. Virus was added to human normal pooled plasma, where plasma clotting time can be measured with the addition of 5 mM calcium ions. As factor VIII is responsible for another FX activating pathway, factor VIII deficient plasma was also tested and factor VIII function was restored with the addition of recombinant factor VIII. A soluble form of gC was expressed in a baculovirus system and purified using a histidine tag. The ability of the soluble gC to participate in FX activation was tested using virus or relipidated recombinant human TF (Dade® Innovin®) in a FX chromogenic assay.

Results:
My results have shown that virus with gC enhanced FXa generation compared to without gC, but FX activation required TF presence on the virus. In addition, FX activation by HSV1 TF or recombinant human TF was accelerated with the addition of soluble gC. This enhancement was largely dependent on the levels of FX rather than factor VIIa. In plasma, gC enhanced TF-dependent clotting times induced by HSV1, although recombinant factor VIII compensated for the absence of gC.

Conclusions:
HSV1 gC accelerates FX activation in a TF-dependent manner. This enhancement of the TF-dependent pathway also occurs in plasma. Furthermore, a soluble form of gC increased FX activation by TF in a FX-dependent manner, lending to the procoagulant role of the virus protein. Our work will advance a general model applicable to all enveloped viruses that may propagate in TF-bearing cells and consequently predispose an individual to hypercoagulability leading to vascular disease.

JESSICA MORRICE

Supervisor: Dr. Christopher Shaw, Dr. Cheryl Gregory-Evans
Session: Basic Sciences

"Timeline of Asymmetrical and Symmetrical Motor Neuron Loss in a Zebrafish Model of Amyotrophic Lateral Sclerosis"

AUTHORS:
Jessica R. Morrice[1], Cheryl Gregory-Evans[1,2], Christopher A. Shaw[1,2]

AFFILIATIONS:
[1]Department of Pathology, University of British Columbia; [2]Department of Ophthalmology, University of British Columbia

Background/objectives:
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder more recently described as a non-cell-autonomous disease depicted by the progressive degeneration of upper and lower motor neurons. Most patients display distinct unilateral neurodegenerative symptoms, which progress bilaterally throughout disease progression. An unexplored area in the field of ALS research is the asymmetrical onset of motor loss displayed in ALS patients at a cellular level. ALS pathology displays simultaneous motor neuron degeneration and central nervous system (CNS) inflammation, and microgliosis has been shown to directly contribute to the degenerative process. Evidence suggests that microglia have a differential phenotype during neurodegeneration, where they present a protective phenotype during initial stages of disease and change to a degenerative phenotype in later stages. However, the inherent features of microglial-neuronal interactions during ALS pathogenesis remain poorly understood.

Zebrafish have emerged as an attractive model for ALS pathology because they are easily genetically manipulated and optically transparent as embryos, and have a similar yet simplified nervous system to humans which is invaluable for in vivo imaging at various stages of disease phenotype. This project aims to investigate the spatiotemporal process of motor neuron degeneration during pre-symptomatic and early stages of neurodegeneration involved in both a genetic and a sporadic model of ALS. We will test the interrelated hypotheses that: There will be naturally pre-existing asymmetries in the distribution of resident microglia within the CNS which will predict any asymmetry in neurodegeneration during pre-symptomatic stages in both the genetic and sporadic disease models. To do this, we have outlined two specific aims: (1) to determine at which stage of disease colocalization initiates between motor neuron apoptosis and microgliosis and (2) to determine if a spatial pattern exists where motor neurons begin to apoptose, and if a similar pattern exists for microglia proliferation and phenotype.

Methods:
To do this, neuronal apoptosis and microglia-tagged double transgenic zebrafish embryos will be used to monitor microgliosis and neuronal cell death spatiotemporally in vivo to create a "map" of neurodegeneration in ALS. To model a genetic ALS phenotype, a morpholino will be used to knockdown C9orf72 in embryos. To model the sporadic ALS phenotype, embryos will undergo ketamine treatment. Colocalization interactions between microglia and neuronal cell death in both ALS phenotypes will be visualized at various early stages of disease by live imaging under the confocal microscope. Motor axons and microglia phenotype will be analyzed by whole mount immunohistochemistry.

Results:
We expect that any asymmetry in presentation of initial disease phenotype is primarily due to pre-existing asymmetry in resident microglia, and that microglia phenotype will directly dictate motor neuron susceptibility to degeneration.

Conclusions:
This research could provide insight into cellular mechanisms involved in the neurodegenerative process and also for investigating motor neurons that are susceptible to degeneration in ALS.

YE QIU

Supervisor: Dr. Decheng Yang
Session: Basic Sciences

"Cleavage of nfat5 During Coxsackievirus Infection Enhances Virus-induced Cell Death"

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

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Centre for Heart Lung Innovation, UBC and St. Paul's Hospital

Background/objectives:
Coxsackievirus B3 (CVB3) is a predominant pathogen of viral myocarditis, an inflammatory disease of the myocardium. CVB3 infection induces cellular apoptosis, which is one of the major causative factors leading to the damage of myocardium and is also important for CVB3's own replication. However, CVB3 infection activates p38 MAPK, which is generally related to anti-apoptosis. Nuclear factor of activated T-cells 5 (NFAT5) is a downstream signal protein induced by activated p38 MAPK. NFAT5 was reported to relieve cellular apoptosis and inflammation induced by hyperosmolar stress via stimulating the expression of chaperone protein Hsp70-2 and inhibiting inflammatory cytokines. Previous studies showed that knocking-down of NFAT5 exaggerated apoptosis regardless of high p38 MAPK activity. According to bioinformatic prediction, several sites on NFAT5 may be potentially cleaved by 2A, a viral protease of CVB3. Hence, inactivation of NFAT5 via cleavage may be closely related to CVB3-induced cellular apoptosis and inflammation of myocardium. Therefore, we frame our hypothesis that NFAT5 is cleaved and inactivated by CVB3 protease 2A during viral infection and inactivation of NFAT5 enhances viral replication and virus-induced cell apoptosis.

Methods:
HeLa cells expressing N-FLAG-NFAT5 were infected by CVB3 and cleavage of the recombinant NFAT5 was determined by anti-FLAG antibody. Full amino acid sequence was analyzed by NetPicoRNA 1.0 to find potential cleavage sites of 2A protease. Then those sites were mutated individually by plasmid mutagenesis and screened by 2A cleavage assay using lysates extracted from CVB3-infected HeLa cells.

NFAT5 was overexpressed by plasmid transfection or knocked-down with siRNAs in CVB3-infected HeLa cells. Cellular apoptosis was detected by MTS cell viability assay and caspase-3 cleavage assay. Meanwhile, CVB3 replication was evaluated by Western blot detecting viral capsid protein VP1.

Results:
Full-length of N-FLAG-NFAT5 with a molecular weight of ~200 kDa disappeared gradually during CVB3 infection and a smaller FLAG-tagged fragment of ~75kDa was detected. NFAT5 mutant G503A was not reduced during CVB3 infection and no fragment of 75kDa was detected. Overexpression of NFAT5 increased cell viability and reduced VP1 level in CVB3 infection, while knocking-down of NFAT5 resulted in an increase of VP1.

Conclusions:
NFAT5 is cleaved by CVB3 protease 2A at the site of G503; such cleavage enhances CVB3 replication and CVB3-induced cellular apoptosis.

SARA SABERI

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Longitudinal Dynamics of Leukocyte Telomere Length (LTL) in a Cohort Study of Pregnant Women: Shorter LTL Associated with HIV and Smoking"

AUTHORS:
Sara Saberi[1,4], Beheroze Sattha[1], Arianne Y.K. Albert[2], Evelyn J. Maan[3], Julie van Schalkwyk[1,2], Deborah M. Money[1,2,3], Hélène C.F. Côté[1,2,3,4] and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Women's Health Research Institute; [3]BC Women's Hospital and Health Centre; and [4]Centre for Blood Research

Background/objectives:
Women of childbearing age constitute around 50% of HIV+ people worldwide. While 90% of HIV+ children acquire the virus through their mother, treatment with combination antiretroviral therapy (cART) during pregnancy and delivery can reduce vertical transmission from around 25% to <2%. CART, HIV proteins, and chronic inflammation/oxidative stress can all potentially affect telomerase activity and/or leukocyte telomere length (LTL), a marker of aging and predictor of lifespan, and shorter LTL was reported in HIV+ adults. However, the effect of pregnancy, HIV and cART use during pregnancy on LTL dynamics remains unclear. We measured LTL in HIV+ and HIV- pregnant women and investigated the predictors of shorter LTL in this context.

Methods:
Peripheral blood, demographic, clinical and substance use data were collected from HIV+ (n=107) and HIV- (n=68) pregnant women enrolled in a prospective cohort study. A multiplex qPCR assay was used to measure relative LTL at three visits during pregnancy (visit A: 13-23, visit B: >23-31, and visit C: >31-40 weeks of gestation). For HIV+ women, visits at delivery and 6 weeks post-partum also took place. Mixed-effects linear regressions were used to examine the univariable relationship(s) between LTL and ethnicity, HIV status, hepatitis C virus (HCV), substance use throughout pregnancy, gestational age (GA) at visit, and preterm delivery (GA<37 weeks). Predictors significantly associated with LTL were included in multivariable analysis. Among HIV+ women, CD4 nadir, CD4 and HIV plasma viral load (pVL) and cART status (on/off) at visit, highest HIV pVL on record, duration of cART exposure in pregnancy, cART regimen type, number of cART interruptions >1 week during pregnancy, and time of visit were also considered.

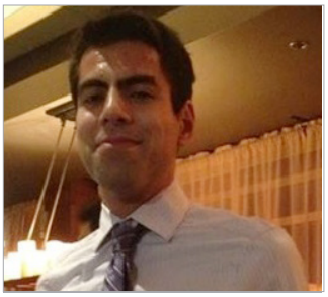
Results:
HIV+ and HIV- women were similar in age (31±6 vs. 31±5 years, p=0.49), but there were fewer Black/African Canadians and HCV+ women in the HIV- group (p<0.001). Twenty-six (26) % of HIV+ women started cART pre-conception (all others initiated treatment during pregnancy), and 61% continued cART post-partum. Univariately, LTL was significantly shorter in HIV+ women (p=0.02) and smokers (p=0.01). Multivariately, only smoking remained associated with shorter LTL (p=0.02). However, a significant interaction between HIV status and GA at visit was observed (p=0.01), suggesting that LTL remained largely unchanged during pregnancy in HIV- women, but increased over time in HIV+ women. Among HIV+ women, smoking, higher HIV pVL, and being off cART at visit were univariately associated with shorter LTL (p≤0.001). In addition, LTL at visit B and C was significantly longer than visit A and post-partum visit (p≤0.03). Multivariately, smoking (p=0.005) and being off cART (p<0.001) remained independently associated with shorter LTL.

Conclusions:
In the context of pregnancy, HIV+ status and smoking are associated with shorter LTL. Within HIV+ women cART initiation may increase LTL, possibly via reduced inflammation and oxidative stress. Whether shorter LTL at post-partum is related to pregnancy ending or HIV/cART is unclear as post-partum samples were unavailable in controls.

ARASH SAMIEI

Supervisor: Dr. Poul Sorensen
Session: Basic Sciences

"Requirement of Eukaryotic Elongation Factor 2(eEF2K) to Induce Activation of DNA Damage Response in Response to Genotoxic Stress"



AUTHORS:
Arash Samiei[1,2], Gabriel Leprivier[1], Poul H. Sorensen[1,2]

AFFILIATIONS:
[1]Department of Molecular Oncology, British Columbia Cancer Research Centre;
[2]Pathology and Laboratory Medicine, University of British Columbia

Background/objectives:
Preserving genome integrity is of utmost importance for maintaining cell viability however the most effective cancer therapy methods induce DNA damage, including chemotherapeutic drugs and radiation. Cells employ a wide variety of mechanisms to help detect and repair DNA damage or to regulate cell cycle progression and induce cell apoptosis if the damage is significantly high. Ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) are major DNA damage response (DDR) transducer proteins that allow for DNA repair, cell cycle regulation, and apoptosis through their downstream proteins which include Chk-1, Chk-2, and P53. However it is not very clear how ATM and ATR are activated after DNA damage. Eukaryotic elongation factor 2(eEF2K) is a regulator of translation that inhibits eEF2 during the elongation phase of protein translation. It has been observed that eEF2K sensitizes cells to hydrogen peroxide, an inducer of DNA damage. We intend to define the mechanistic role of eEF2K in DDR and its role in the activation of the ATM/ATR pathway in response to genotoxic agents and irradiation. We hypothesize that eEF2K helps to activate the ATM/ATR DDR pathways in response to genotoxic agents and radiation.

Methods:
Wild type (WT) and eEF2K knockout (KO) mouse embryonic fibroblast (MEFs) were treated with cisplatin, irradiation, and doxorubicin. Trypan blue and MTT assays were carried out on cisplatin treated cells to quantify the cell viability and cell death off eEF2K KO and WT MEFs. Immunoblot analysis of the cells was done to view the DDR proteins and their activation. To visualize localization of DDR proteins the gH2AX and 53BP1 foci were quantified through immunofluorescence microscopy in the WT MEFs and eEF2K KO MEFs treated with cisplatin.

Results:
Our results show that eEF2K expressing MEFs are less viable and more sensitive to cisplatin treatment when compared to the eEF2K KO MEFs. There was a lower activation of the proteins in the ATM/ATR pathway when treated with cisplatin, doxorubicin, and IR, indicating that the role eEF2K in DDR is not limited to the type of DNA damage. The proteins that had reduced phosphorylation in the eEF2K KO MEFs included ATM, ATR, and H2AX indicating that the role of eEF2K is upstream of these early DDR proteins. eEF2K expression also has an effect on the effector proteins in the DDR pathway, since the eEF2K KO MEFs had reduced activation of cell cycle and apoptosis regulatory proteins (Chk1 and P53) in response to doxorubicin, cisplatin, and IR treatment. When treated with cisplatin there was reduced gH2AX and 53BP1 foci localization in the eEF2K KO MEFs compared to the eEF2K WT MEFs, illustrating that there is reduced localization of DDR proteins to the site of damage.

Conclusions:
We illustrated that eEF2K sensitizes cells to cisplatin treatment and it plays a prominent role in the DDR where eEF2K is required to induce the ATM/ATR pathway. Furthermore eEF2K could also play a role in localization of the DNA damage proteins after cisplatin treatment.

MEHUL SHARMA

Supervisor: Dr. David Granville
Session: Basic Sciences

"Extracellular Granzyme K Mediates Endothelial Activation Through the Cleavage of Protease Activated Receptor-1 (PAR-1)"

AUTHORS:
Mehul Sharma[1], Sheetal Raithatha[1], Leigh G. Parkinson[1], Steve Shen[1], Yulia Merkulova[1], Dawn M. Cooper[2], and David J. Granville[1]

AFFILIATIONS:
[1]Centre for Heart Lung Innovation and Department of Pathology and Laboratory Medicine, UBC; [2]Department of Cellular and Physiological Sciences, UBC

Background/objectives:
Microbial infections have the potential to cause wide-spread inflammatory immune responses or sepsis. The complex pathology of sepsis prevents its resolution using simple measures, with anti-microbial drugs proving ineffective. Endothelial dysfunction and increased endothelial permeability are thought to play an important role in the pathogenesis of sepsis. Recently the serine protease, Granzyme K (GzK), was observed to be significantly elevated in serum from patients with sepsis while its inhibitor, IαIp, was found to be reduced. As such, the role of GzK in Protease Activated Receptor (PAR) activation was investigated. Previous studies have shown that PAR activation triggers downstream signalling for cytokine production, cell proliferation, migration and extracellular matrix synthesis. In addition, we have previously shown that extracellular GzK activates PAR-1 and its downstream inflammatory cascade in lung fibroblasts. We hypothesize that elevated levels of GzK modulates endothelial activation/dysfunction through the activation of PAR-1.

Methods:
The effects of extracellular GzK on HUVECs were assessed by treating monolayers of HUVECs with GzK (1nM - 50nM). BrDu assay was performed to measure cell proliferation, and ELISAs was used to study cytokine production of MCP-1 and IL-6. Cell lysates were used to study the intracellular signalling pathway via western blot. Inhibitor assays were performed to confirm these findings. Endothelial permeability was measured via trans-endothelial electrical resistance assays using ECIS (Electric Cell-substrate Impedance Sensing).

Results:
GzK cleaved PAR-1 on HUVECs and subsequently induced a pro-inflammatory response. This response was mediated via the MAPK pathway, p38 and ERK1/2, no evidence of NF-kB activation was seen. The expression of adhesion molecules (ICAM-1, VCAM-1 and E-selectin) was upregulated upon GzK treatment, and an increase in THP-1 adherence to GzK-treated HUVECs was also seen. HUVECs primed with TNFα and subsequently treated with GzK had enhanced production of cytokines and expression of adhesion molecules. These responses were abolished by the physiological inhibitor of GzK, IαIp. GzK treatment did not result in any cell toxicity, or cause endothelial permeability across the monolayer.

Conclusions:
In summary, our results suggest that extracellular GzK induces pro-inflammatory cytokine expression through the cleavage and activation of PAR-1. Further research into the role of GzK in sepsis and endothelial activation are needed to support GzK as a novel therapeutic target for sepsis treatment.

KAREN SIMMONS

Supervisor: Dr. Soren Gantt
Session: Basic Sciences

"Characterizing Early Events During Primary Acquisition of Gammaherpesvirus in Mice"

AUTHORS:
Karen Simmons[1,2] and Soren Gantt[1,2,3]

AFFILIATIONS:
[1]Child and Family Research Institute; [2]Department of Pathology and Laboratory Medicine; [3]Department of Infectious and Immunological Diseases (Pediatrics)

Background:
Epstein-Barr Virus (EBV), a gammaherpesvirus, infects nearly everyone at a young age. Primary infection is typically asymptomatic, however it can result in infectious mononucleosis. Additionally, chronic EBV infections can lead to the development of different lymphomas and carcinomas. There is no vaccine against EBV, and a further understanding into the mechanisms of acquisition and establishment of primary infections is needed for vaccine development. It is thought that acquisition of EBV occurs though inoculation of the mouth where it undergoes lytic replication in oral epithelial cells. Following lytic replication, EBV establishes a life-long latent infection in B cells. Previously, a cohort study was carried out following 32 infants in Uganda to characterize primary herpesvirus infection. This study revealed that many infants would have a local infection where viral genome was present in oral swabs at low levels that did not result in a primary infection, referred to as "blips". This suggests that EBV replication in oral epithelium does not always lead to life-long latent infection. Additionally, mathematical modelling of this cohort data suggests that only approximately 20% of all inoculation events actually result in a primary infection.

Objectives: To model the "blips" seen in the cohort study, by using the mouse model of EBV, murine gammaherpesvirus 68 (MHV68). To investigate the kinetics of primary infection by determining the infectious dose (ID) at which 50% of mice are reliably infected with MHV68 (ID50). Finally, to determine the ID at which local lytic viral replication occurs without resulting in life-long latent infection. **Hypothesis:** There will be an infectious dose at which localized lytic replication of MHV68 occurs, without subsequent latent infection.

Methodology and Preliminary Results:
Viral presence and replication will be investigated via quantitative (q)PCR and reverse transcriptase (RT)-qPCR, respectively. Primers have been designed within the lytically-expressed rta gene. The RTA transcript has a splice site, at which transcript specific primers have been designed. For genomic detection, primers are within the intron of the rta gene. This will ensure specific amplification of the desired RNA or DNA product. Preliminary results suggest that these primers function as expected using SYBR® Green fluorescent DNA dye. In the future amplicon specific probes will be used to ensure specificity and to increase sensitivity of the assay. Once the qPCR assays are complete, an in vivo MHV68 infection model will be used to investigate primary gammaherpesvirus infection. Groups of at least 6 mice will be infected through intranasal inoculation with low plaque forming unit (pfu) serial dilutions of MHV68. A nasal swab will be collected every day for 10 days, from which DNA and RNA will be extracted and quantified. The spleens of these mice will then be investigated at 30 days post inoculation for the presence of latent MHV68 infection.

Expected Results: Using the primers with probes specific to rta gene or RTA lytic viral transcript, the ID50 will be determined. At low dose inoculation, local lytic replication of MHV68 will occur in mice nasal mucosa without development of a systemic infection leading to life-long latency.

Significance: This model will effectively mimic the "blips" seen in the cohort data, allowing further investigations into the mechanisms and kinetics of primary gammaherpesvirus infections. A greater understanding of the biology of early events in EBV infection holds implications for vaccine design or other preventative advances against primary herpesvirus infections.

NICHOLAS SWYNGEDOUW

Supervisor: Drs. Chun Seow & Peter Paré
Session: Basic Sciences

"Mechanical Properties of Airway Smooth Muscle Tissue Stimulated with the Inflammatory Mediator Interleukin-13"

AUTHORS:
Nicholas E. Swyngedouw[1,2], Christopher D. Pascoe[2], Lu Wang[2], Peter D. Paré[2,3], Chun Y. Seow[1,2]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Centre for Heart Lung Innovation, St. Paul's Hospital; [3]Department of Medicine, Respiratory Division, University of British Columbia

Background/objectives:
Asthma is an obstructive airway disease that affects an estimated 300 million people worldwide. Airway smooth muscle (ASM) contributes to the excessive airway narrowing that causes many symptoms of asthma. ASM dysfunction, together with airway inflammation, has also been suggested as a mechanism for airway hyperresponsiveness (AHR) in asthmatics. Inflammatory mediators (IMs) known to be upregulated in the sera of asthmatics have been reported to directly affect ASM cells by altering their contractile or proliferative phenotype in vitro. A recently proposed concept suggests that it is the inflammatory milieu, or environment, of the ASM cells alters their function in asthma and induces intracellular changes. Stimulation of non-asthmatic ASM with IMs may explain how the in vivo environment of asthmatic ASM contributes to their phenotype and potential hypercontractility. So far, little is known about how these inflammatory mediators influence the mechanical properties of ASM tissues. In this investigation we examine the influence of the inflammatory cytokine interleukin-13 (IL-13) at a concentration found in the serum of acute asthmatics, on the force production and shortening velocity of isolated ASM tissue. The hypothesis for this project is that ASM tissue incubated with IL-13 will generate increased force and shortening velocity in response to the contractile agonist acetylcholine (ACh) relative to control tissues not exposed to IL-13.

Methods:
To evaluate the effect of IL-13 on ASM tissue mechanics, tissue strips at in situ length were incubated for 72h with or without IL-13 in serum-free culture media. Tissues were stimulated with ACh to get baseline (t=0) and post-incubation (t=72h) measurements of maximal force and shortening velocity against a load which was 10% of Fmax. At t=72h mechanical data was compared to values obtained at t=0. Western blot analysis was conducted to investigate the phosphorylation of STAT6 in response to IL-13.

Results:
After 72 hours, all tissues remained viable and contracted in response to ACh. ASM tissue exposed to IL-13 showed a similar modest increase in maximal force compared to control. Likewise, ASM tissue exposed to IL-13 showed a small decrease in shortening velocity which was comparable to the decrease seen in the control non-exposed tissue. Phosphorylated STAT6 (pSTAT6), downstream of IL-13R, was detected by Western Blot of IL-13 treated ASM tissue and not in control.

Conclusions:
IL-13, at a concentration similar to that observed in serum during an acute asthmatic attack, is able to induce intracellular changes within ASM tissue via pSTAT6. However, IL-13 does not appear to cause increased force generation or shortening velocity in ASM tissue. Further investigation is required to elucidate if, and how, IL-13 can influence the mechanical properties of ASM. Understanding of the role of ASM contractility in AHR can impact the development of therapeutic interventions to alleviate asthma symptoms.

BRENNAN WADSWORTH

Supervisor: Dr. Kevin Bennewith
Session: Basic Sciences

"Detection of Transient Tumour Hypoxia with a Novel Combination of Oxygen-sensitive Fluorescent Proteins"

AUTHORS:
Brennan J Wadsworth, Elizabeth C Halvorsen, Kevin L Bennewith

AFFILIATIONS:
BC Cancer Research Centre

Background/objectives:
Solid tumours are understood to develop regions of low oxygen, or 'hypoxia'. Hypoxia in tumours is associated with resistance to chemotherapy and radiotherapy. Detection of hypoxia in tumours clinically and experimentally has been accomplished using exogenous markers or via detection of proteins produced by cells experiencing hypoxia. These methods are sufficient to assess cross-sectional prevalence of hypoxia. However, recent evidence has revealed that hypoxia occurs in tumours as short-lived cycles, referred to as transient hypoxia. This phenomenon is hypothesized to be dependent on perfusion changes. Unfortunately, challenges with detecting transient hypoxia have resulted in poor characterization and clinical consideration. An experimental method to detect transient hypoxia could lead to future development of techniques to identify patients with hypoxic tumours, which current practice is missing.

I aimed to (1) develop an improved method for experimental detection of transient hypoxia, (2) detect transient hypoxia with traditional hypoxia markers for comparison with my novel method, and (3) provide evidence for perfusion changes in tumours.

Methods:
1) I designed two vectors to be used together encoding either green or red fluorescent proteins. A hypoxia response element was the driver of expression for both fluorescent proteins. I added an oxygen dependent degradation domain to the green protein to rapidly degrade its signal upon reoxygenation, while the red protein was expected to degrade at a slower rate. Vectors were transfected into cells and subjected to time course hypoxia and reoxygenation assays to determine the kinetics of signal induction upon hypoxic exposure and degradation upon reoxygenation.

(2,3) *In vivo* experiments were conducted with human tumour xenografts grown in NOD SCID mice. The ability of exogenous markers to detect changes in tumour hypoxia was tested by administering markers either two or four hours apart, with a similar protocol for perfusion markers. Immunofluorescent images were produced to analyze tumour hypoxia and perfusion.

Results:
Vectors for fluorescent proteins were successfully constructed and transfected cells characterized for hypoxic induction and oxygen-dependent degradation kinetics. Threshold analysis of immunofluorescent images from tumours reliably revealed areas of transient hypoxia with the two and four hour separation between marker exposures. Changes in regional tumour perfusion were also detected with two and four hour marker separations.

Conclusions:
Our results support that the fluorescent protein combination will be capable of detecting transient hypoxia in experimental models and that we will be able to relate this to perfusion changes. Future studies will attempt to modify transient hypoxia with blood flow modifying agents and vascular modifying agents. It will also be possible to conduct improved studies looking for clinically relevant bio-markers.

XINING (LINDA) YANG

Supervisor: Dr. Mark Scott
Session: Basic Sciences

"Anti-Tumor miRNA Therapy in Human Cell Lines and in Mice"

AUTHORS:
Xining (Linda) Yang[1,3], Mark Scott[1,2,3]

AFFILIATIONS:
[1]University of British Columbia, Department of Pathology & Laboratory Medicine; [2]Canadian Blood Services; [3]Centre for Blood Research

Background/objectives:
Tumors are abnormal masses of tissue resulting from excess cell division and have the potential to cause significant morbidity and mortality. Tumoigenesis is closely correlated with ineffective immune recognition of 'non-self' resulting in a poor anti-tumor inflammatory response. The inflammatory response is dependent upon activation and/or proliferation of immune cells such as T cells, B cells, natural killer (NK) cells and dendritic cells (DCs). Recent studies have demonstrated that microRNAs (miRNAs) are key regulators of cellular process including the immune response. miRNAs are small, non-coding RNAs that regulate the expression of genes at the post-transcriptional level and are involved in a wide range of biological responses. Emerging evidence suggests the use of miRNA mimics or anti-miRNAs as therapeutic strategies in inflammatory diseases. However, miRNA regulation is complex and from a bio-regulatory approach, the differential expression of multiple miRNAs are necessary for initiating a biological response. Hence our lab developed a purified miRNA-based cocktail (denoted as Inflammatory Agent 1; IA1) that mimics the 'pattern of miRNA expression' (encompassing increased, decreased and static levels) to achieve pharmacologically effective therapeutics. We hypothesize that our miRNA preparation IA1 will give rise to a systemic pro-inflammatory immune state resulting in the inhibition of tumor growth and metastatic spread. Previous data has demonstrated that IA1 resulted in significant ($P<0.001$) increases in pro-inflammatory cells (e.g., NK cells) in vivo. To manufacture standardized IA1 using a bioreactor approach in vitro, our initial objective has been to confirm the allorecognition-stimulating effect of "*unprocessed IA1*" containing the miRNAs and optimize the production of IA1 from human peripheral blood mononuclear cells (PBMCs).

Methods:
"*Unprocessed IA1*" – IA1 enriched conditioned media was prepared in vitro using mixed lymphocyte reactions (MLR). The pro-inflammatory efficacy of "unprocessed IA1" was analyzed by lymphocyte proliferation. PBMCs were isolated from whole blood using Ficoll-Paque PREMIUM from two HLA-disparate human donors. Conditioned media from primary (1°) 2-way MLR was collected at Day 1, 3, 5 and 7 for conducting a secondary (2°) MLR. The 2° MLR utilized freshly prepared PBMCs from the same or different donors as the initial plate. Following 13 days of incubation (37°C, 5% CO2), cells were harvested and cell proliferation was measured via flow cytometry using the CellTrace CSFE Cell Proliferation Kit.

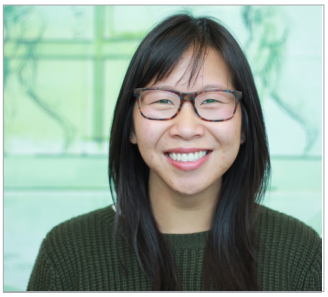
Results:
The human MLR model mimics the allorecognition of cancer cells based on identification of 'non-self' signals in vitro. In this study, "*unprocessed IA1*" enhanced an inflammatory response by increasing allorecognition-mediated lymphocyte proliferation. The index of increase in lymphocyte proliferation was correlated with the time point of collecting "*unprocessed IA1*".

Conclusions:
We have confirmed that "*unprocessed IA1*" is capable of enhancing an inflammatory response, similarly to purified IA1 that mediates the complex patterns seen in allorecognition in vitro. Subsequent studies will focus on the manufacturing of miRNA-concentrated IA1 using the human MLR model. Compositional studies will also be conducted for a purpose of standardized production of IA1.

ADA YOUNG

Supervisor: Dr. Kevin Bennewith
Session: Basic Sciences

"Radiation Enhanced Migration and Metastasis of Breast Cancer in Co-culture Studies"



AUTHORS:
Ada Young[1,2], Kevin Bennewith[1,2]

AFFILIATIONS:
[1]Integrative Oncology Department, British Columbia Cancer Agency; [2]Department of Pathology and Laboratory Medicine, University of British Columbia

Background/objectives:
In Canada, cancer remains the leading cause of mortality with metastasis as the leading contributor to this mortality rate. Metastasis occurs when cancer cells dissociate from the primary tumour mass, escape into the lymphatic or blood system, and re-establish a secondary mass in sites like the lungs and brain. Recent studies have suggested that radiation therapy, although an efficacious treatment for patients with localized primary tumours, has the potential to promote metastasis in patients post-treatment. Several studies have suggested that ionizing radiation may promote tumour cell migration, although the majority of these studies have been done in vitro, using plastic plates or porous membranes. The effect of radiation on tumour cells has not been studied in an environment where they directly interact with cells of their stromal environment. Fibroblasts are host cells in the tumour microenvironment, secreting extracellular matrix, growth factors and cytokines that support tumour growth. Similarly, in vivo co-injection studies using cancer lines and primary fibroblast cells have been conducted to study metastasis, but the effect of radiation treatment on metastasis has not been well characterized. We hypothesize that breast cancer cells co-cultured with fibroblasts, when irradiated, will exhibit increased migration and/or invasion in vitro and an increased frequency of lung metastases *in vivo*.

Methods and Results:
Breast cancer cell lines and fibroblasts stably expressing EGFP and mCherry were produced via lentiviral transduction. Fibroblast cells were exposed to doses of radiation and we found that 40Gy was the lowest dose at which cells senesced but no major cell death was observed (which is important for co-culture studies to prevent the fibroblasts from overtaking the culture). We have also assessed the radiation response of the tumour cell lines in preparation for our co-culture studies. We will culture breast cancer cell lines with/without fibroblast lines for analysis with the IncuCyte ZOOM system. This will allow for in vitro real-time measurement of changes in migration and/or invasion of breast cancer cells when in direct interaction with fibroblasts, post radiation treatment. We will implant tumour cells expressing EGFP or mCherry, with/without fibroblasts, subcutaneously in NOD/SCID mice and treat the mice with radiation. Following treatment, mice will be sacrificed and the lungs and tumour will be resected for immunohistochemistry, flow cytometry or for clonogenic assay to determine changes in migration and/or invasion.

Significance:
It is evident that the cross-talk between cancer cells and cells of the tumour microenvironment not only defines the progression of the disease but also plays a large role in treatment outcome. By elucidating the changes when the primary tumour and its stroma are exposed to radiation, I will explore the magnitude of radiation-induced metastasis. These findings will help alert clinicians to the potential risk of using radiation treatment alone and to look to other adjuvant therapies, perhaps in combination with radiation, to reduce or eliminate the possibility of metastasis.

ADAM YU

Supervisor: Dr. Jacqueline Quandt
Session: Basic Sciences

"Examining DNAJC13 Expression within the Neurovascular Niche and its Role in the Pathogenesis of Inflammatory Neurodegenerative Disease"

AUTHORS:
Adam Yu[1], Sarah Neil[1], Jacqueline Quandt[1]

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

Background/objectives:
Multiple sclerosis (MS) is a disease of the central nervous system that can result in vision and speech impairment, cognitive dysfunction, and paralysis. DNAJC13 is a protein that regulates vesicular trafficking at the level of endosomal cycling, thereby influencing cell function. In a subset of hereditary adult onset autosomal dominant Parkinson Disease, DNAJC13 was found to be the common mutated gene amongst family members. Findings suggested DNAJC13 may have a greater role in neurodegenerative processes where trafficking with retromer proteins are key to axonal survival and function. Our study aims to characterize the functional role of DNAJC13 in neuroinflammatory and degenerative processes at the neurovascular niche.

Methods:
Primary murine cortical neurons, astrocytes, and the human cerebral microvascular endothelial cell line (hCMEC)/D3 were cultured per previously established protocols. Our laboratory developed a novel protocol to prepare high numbers of microglial cells possessing several characteristics of their in vivo counterparts. DNAJC13 expression was examined using immunoblotting and immunocytochemistry. Immunohistochemistry was used to localize and quantify DNAJC13 in the brain and spinal cord tissue of healthy animals compared to clinically disabled animals undergoing disease secondary to immunization for experimental autoimmune encephalitis, the murine model of MS.

Results:
Western blot analysis of primary cell cultures showed that microglia had constitutively the highest relative expression of DNAJC13, two fold higher than the next highest expressing cell, the astrocyte. Microglial expression was 2.5 and 5 times greater than the hCMEC/D3 cell line and cortical neurons respectively. Animals that displayed no clinical symptoms with sparse inflammatory infiltrates showed relatively low DNAJC13 expression in both astrocytes and microglia throughout the spinal cord. Motor neurons had the highest expression of DNAJC13, with intense nuclear and perinuclear staining throughout the dorsal and ventral lateral horns. Disabled animals with high levels of inflammatory infiltrates throughout the white matter showed increased DNAJC13 expression associated with infiltrating macrophages and microglia. We are currently investigating alterations in neuronal expression of DNAJC13 in this inflammatory setting. Ependymal cells lining the central canal were found to have a relatively high level of constitutive expression of DNAJC13.

Conclusions:
DNAJC13 expression in vivo by cells of the neurovascular niche was found to be modeled well in our *in vitro* systems. Comparison of non-diseased to diseased tissues with high lesion load showed significant changes in DNAJC13 expression, and presumably also cellular function, which may contribute to disease. Future experiments aim to characterize the functional role of DNAJC13 in these pathologic processes and to examine the potential of targeting this pathway to improve axonal stability, limit disease severity and progression in MS, which may enhance our understand of other neurodegenerative processes and disorders.

NOORAH ALMADANI

Supervisor: Dr. Jefferson Terry
Session: Basic Sciences

"Autopsy Findings in RMND1 - Related Mitochondrial Cytopathy"

AUTHORS:
Noorah Almadani[1,2], Glenda Hendson[3], Hilary Valance[3], Jefferson Terry[3]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British;
[2]Department of Pathology and Laboratory Medicine, King Abdulaziz Medical City and National Guard Hospital, Riyadh, Saudi Arabia;
[3]Department of Pathology and Laboratory Medicine. Children's and Women's Health Centre of British Columbia

Background/objectives:
RMND1 encodes a protein that localizes to the inner mitochondrial membrane where it plays a role in translation of the 13 mtDNA encoded polypeptides which are all structural subunits of the respiratory chain complexes. [1]. Mutations in RMND1 lead to development of a mitochondrial cytopathy characterized by lactic acidosis, deafness, renal dysfunction and myopathy [2-4]. Renal dysfunction is a relatively uncommon feature of mitochondrial disease suggesting RMD1 may preferentially affect kidney function. Presently there are no detailed published descriptions of the autopsy findings associated with RMND1 related mitochondrial cytopathy.

Methods:
Herein, we report the autopsy findings in a 4-year-old boy with mitochondrial cytopathy caused by pathogenic mutations in RMND1. Full clinical and biochemical features of the index case were recently published [3]. Briefly, he was born at term following a pregnancy complicated by oligohydramnios. Mild sensorineural hearing loss was detected at birth through routine newborn screening. At 2 months of age, he presented with failure to thrive, diarrhea and epileptic encephalopathy. His unusual clinical renal presentation included lactic acidosis and renal dysfunction (declining glomerular filtration rate, hypertension, hyperkalemia, and hyponatremia). Psychomotor development was delayed with severe hypotonia and myopathic features. Skeletal muscle complexes I and IV were significantly reduced. Magnetic resonance imaging (MRI) revealed increased T2 signals throughout the white matter and delayed myelination. His clinical condition deteriorated and he expired primarily due to progressive chronic renal failure at 4 years of age.

Results:
At autopsy, renal abnormalities were noted including renal hypoplasia, diffuse glomerulosclerosis, tubular atrophy, calcification, interstitial fibrosis, and inflammation. The liver was enlarged secondary to steatosis. Histochemical analysis of skeletal muscle showed diffuse reduction in cytochrome C oxidase activity, abnormal NADH, SDH and PAS staining, increase in lipid and mild type-2 fiber atrophy. Non-specific chronic pancreatitis and adrenal cortical lipid depletion were also present. The spleen showed unusual subintimal deposits in the arterioles associated with the periarteriolar lymphoid tissue; this histological finding was not seen elsewhere. Ultrastructural analysis of kidney and skeletal muscle demonstrated rectilinear electron dense mitochondrial inclusions.

Conclusions:
This case report is the first detailed description of anatomical abnormalities associated with the RMND1 mitochondrial cytopathic phenotype, which is of value to recognizing RMND1 related mitochondrial cytopathy at autopsy and further characterizing the pathophysiology of this abnormality.

SIMA ALLAHVERDIAN

Supervisor:
Session: Basic Sciences

"Smooth Muscle Cells Origin of Foam Cells in Human Atherosclerotic Lesions"

AUTHORS:
Sima Allahverdian[1], Véronique Ollivier[2], Cyrus Chehroudi[1], Thomas Abraham[1], Bruce M. McManus[1], Jean-Baptiste Michel[2], and Gordon A. Francis[1]

AFFILIATIONS:
[1]Centre for Heart Lung Innovation and Department of Medicine, University of British Columbia; [2]DRCE INSERM, Laboratory for Vascular Translational Science, Bichat Hospital, Diderot University, Paris, France

Background/objectives:
Cholesterol accumulation in atherosclerotic plaque has previously been thought to occur primarily in monocyte-derived macrophages. Using coronary artery sections from hearts explanted at time of heart transplantation we have determined the relative contribution of smooth muscle cells (SMCs) to foam cell formation.

Methods and Results:
Lipids in formalin-fixed coronary artery tissues were preserved for staining in paraffin sections, followed by immunohistochemical staining with SM α-actin and Oil Red O. Studies of coronary artery lesions with a high content of foam cells show that at minimum, SMCs comprise 50±7% (Avg±SEM, n=14 subjects) of foam cells in human coronary atherosclerosis. Further estimation of plaque foam cell content using fluorescence-activated cell sorting suggests the contribution of SMCs to foam cells in human atherosclerosis may be much higher. We also found that SMCs in advanced lesion intima have a specific reduction in ABCA1 expression not seen in early or advanced lesion myeloid lineage cells or in early lesion SMCs. These results suggest an inability of SMCs to release cholesterol via the ABCA1-apoAI-HDL axis contributes to the large contribution of SMCs to the foam cell population. To better understand the reasons for cholesterol accumulation in SMCs we apply two approaches. First, combining fluorescence-activated cell sorting of archived human coronary artery specimens with Nanostring gene ncounter analysis we will determine the specific pattern of gene expression in SMC foam cells when compared to macrophage foam cells, and intimal and medial SMCs that do not become foam cells. Second, we have grown SMCs from coronary intima plaque and media. We are testing these cell cultures for gene expression studies with Nanostring gene ncounter analysis, apoA-I binding, and lipid efflux before and after lipid loading.

Conclusions:
The current investigation extend our observations that SMCs comprise a much larger fraction of atheroma foam cells than previously imagined, and explore a likely reason(s) for this finding. It is critical to understand the nature of cholesterol accumulation in SMCs to expand knowledge of the pathogenesis of atherosclerosis, and to define new therapies.

WAI HANG CHENG

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Biomechanical and Functional Characterization of CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) in an APP/PS1 Model of Alzheimer's Disease"

AUTHORS:
Wai Hang Cheng[1], Kristina M. Martens[1], Dhananjay N. Namjoshi[1], Kurt McInnes[2], Peter A. Cripton[2], Cheryl L. Wellington[1]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Departments of Mechanical Engineering and Orthopaedics, University of British Columbia

Background/objectives:
In addition to being a leading cause of disability in young people, traumatic brain injury (TBI) is a risk factor for dementia, including Alzheimer's disease (AD). Notably, both amyloid and tau neuropathology can develop after TBI. We recently developed a novel rodent TBI model called CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) that uses a non-surgical procedure to precisely deliver defined impacts to an intact head with unrestrained and reliable head movement. In C57Bl/6, CHIMERA induces significant behavioral deficits, white matter inflammation, axonal damage and endogenous tau phosphorylation. Here we apply CHIMERA TBI to the APP/PS1 model of AD.

Objectives: To characterize acute biomechanical, behavioral and neuropathological outcomes of repetitive TBI using CHIMERA in APP/PS1 mice.

Methods:
CHIMERA was used to induce two mild TBIs (0.5 J impact energy), spaced 24 hours apart in 5-mo male APP/PS1 mice. Head kinematics were assessed using high-speed videography (5000 fps). Acute behavioral, histological, and biochemical outcomes tests were conducted up to 48h post-injury.

Results:
Head kinematic analysis showed peak displacement of 41.8±3.7 mm, peak angular deflection of 2.3±0.4 rad, peak linear and angular velocities of 5.2±0.4 m/s and 314.0±169.6 rad/s, respectively, and peak linear and angular accelerations of 238.3±79.2 g and 280.3±168.5 krad/s2, respectively. Immediately post-TBI, APP/PS1 mice experienced a prolonged loss of righting reflex compared to sham-operated APP/PS1. Behavioral analysis at 48h revealed increased neurological deficits (neurological severity score), and poorer motor coordination (Rotarod) in injured versus sham APP/PS1. Histological analysis at 48h revealed increased 6E10+ve A-beta deposits, microglial activation (Iba-1), argyrophilic fibre (silver staining), and axonal bulb-like structures (phospho-neurofilaments) in injured versus sham APP/PS1.

Conclusions:
These results support the hypothesis that CHIMERA TBI induces inflammation, white matter pathology, and A-beta deposition in acute post-injury period. Future studies will reveal how CHIMERA TBI affects disease progression in APP/PS1 mice.

JIANJIA FAN

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Identification of a Novel Modulator of apoE in Astrocytes"

AUTHORS:
Shahab Zareyan[1], **Jianjia Fan[1]**, Iva Kulic[1], Yoko Shimizu[2], Tom Pfeifer[2], Cheryl Wellington[1]

AFFILIATIONS:
[1]Djavad Mowafaghian Centre for Brain Health, University of British Columbia; [2]Centre for Drug Research and Development

Apolipoprotien E (apoE) is the most abundant apolipoprotein expressed in the brain, where it is predominantly synthesized and secreted by astrocytes and mediates cholesterol transport in the central nervous system. As APOE is the most highly associated susceptibility locus for Alzheimer's disease, modulating apoE levels or function is of therapeutic interest. To identify compounds that increase secretion of apoE we performed a high throughput screen using a small molecule library of 104,000 compounds in human CCF-STTG1 astrocytoma cells. A hit compound, CD82, belonging to the pyrethroid ester class of insecticides, was confirmed to increase both expressed and secreted apoE up to 6-fold or greater starting at a concentration of 10 micro-M. Upregulation of apoE by CD82 was concomitant with an increase in other liver x receptor (LXR) target genes including LXR-alpha itself, and a robust upregulaton of the lipid transporter ABCA1 (10-fold at 30 μm). As apoE is transcriptionally regulated though the LXR pathway, we evaluated the requirement of LXR function for the observed effect using knockout mouse embryonic fibroblast deficient in LXR-beta or both LXR-alpha and -beta. Induction of both ABCA1 and apoE mRNA expression by CD82 required LXR activity. Importantly, HepG2 liver hepatocellular carcinoma cells were not responsive to CD82 in terms of LXR target gene induction except for a minimal increase in LXR-alpha (1.6-fold) at 30 micro-M. By contrast, a known LXR agonist, GW3965, induced a clear increase in LXR target genes, including the transcription factor SREBP1c, in both CCF-STTG1 and HepG2 cells. Ongoing work is focused on evaluating the mechanisms by which CD82 mediates upregulation of apoE and the functional outcomes.

HUGO HORLINGS

Supervisor: Dr. David Huntsman
Session: Clinical Sciences

" Granulosa-Cell Tumor of the Ovary: a Molecularly Defined Entity"

Background/objectives:
Our understanding of the clinical behaviour of adult granulosa cell tumours (AGCTs) has been confounded by misdiagnosis of cases due to the nonspecific morphologic features. To address this we have studied a large cohort of clinically and molecularly annotated AGCTs.

Methods:
336 AGCT cases from three European centres were subjected to pathology review and tested for the C134W FOXL2 mutation. The clinical outcome of the molecularly defined AGCTs (MD AGCT) as defined by morphology and FOXL2 mutation status was compared to misdiagnosed non-AGCT cases and population based controls.

Results:
Sixty-three (19%) out of 363 cases were reclassified as other tumour types, mainly thecomas and metastatic carcinomas. Of the remaining cases (257/273) 94% were FOXL2 mutation positive MD AGCT and 6% (16/273) were morphologically typical but FOXL2 wild-type AGCTs. Combined cohort analysis showed that the overall survival (HR=5.7) and disease specific survival (HR=8.9) of the misdiagnosed cases was significantly different ($p>0.001$) from the MD AGCTs. 28% of cases relapsed after primary surgery with the medium time to relapse being (7.6) yrs. Population analysis revealed that the overall survival of MD aGCT patients does not differ significantly from age-matched population based controls, even after recurrence.

Conclusions:
Adult granulosa cell tumor of the ovary is characterized by the pathognomonic C134W FOXL2 hot spot mutation, and the clinical course of these molecularly defined cases is indolent Most deaths due to disease in this large cohort were seen in cases misdiagnosed as AGCT, indicating that studies of AGCT from the pre-molecular era must be interpreted with caution.

AUTHORS:
Melissa K. McConechy[2*], Anniina Färkkilä[1*], **Hugo M. Horlings[2*]**, Aline Talhouk[2], Hannah S. van Meurs[3], Noora Andersson[1], Katherina Zaby[4], Winnie Yang[5], Nirit Rozenberg[5], Saara Bryk[1], Leila Unkila-Kallio[1], Ralf Bützow6, Johannes B.G. Halfwerk[7], G.K.J. Hooijer[7], Gemma G. Kenter[3], Stefan Kommoss[4], Maaike C.G. Bleeker[7], Blake C. Gilks[2], David G. Huntsman[2**], Mikko Anttonen[8**]

* /** *These authors contributed equally*

AFFILIATIONS:
[1]Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital; [2]Department of Pathology and Laboratory Medicine, University of British Columbia and BC Cancer Agency; [3]Department of Obstetrics and Gynaecology, Academic Medical Center, Amsterdam, The Netherlands; [4]Department of Obstetrics and Gynaecology, Eberhard Karls Universitats Tübingen, Tübingen, Germany, [5]Centre for Translational and Applied Genomics (CTAG); [6]Department of Pathology, University of Helsinki and HUSLAB, Helsinki University Central Hospital, Helsinki, Finland; [7]Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands; [8]Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

AZADEH HOSSEINI-TABATABAEI

Supervisor: Dr. Bruce Verchere
Session: Basic Sciences

"Mechanism of XIAP-induced Protection of Beta Cells from Autoimmunity"

AUTHORS:
Azadeh Hosseini-Tabatabaei, Merce Obach, Paul C. Orban, Derek Dai, C. Bruce Verchere

AFFILIATIONS:
Child & Family Research Institute, Department of Surgery and Department of Pathology and Laboratory Medicine, University of British Columbia

Background/objectives:
Expression of the X-linked inhibitor of apoptosis (XIAP) in beta cells prevents diabetes in NOD mice and causes a striking dissipation of pancreatic islet infiltration. We found that XIAP increased expression of two repressed genes in beta cells, lactate dehydrogenase (LDH) and monocarboxylate transporter (MCT1), accompanied by islet secretion of lactate, a potent immunosuppressor.

Methods:
To determine whether XIAP induction of islet lactate production can suppress local immunity, we used an adeno-associated viral vector driven by the rat insulin promoter (AAV8-RIP) for beta-cell specific expression of XIAP. To find out whether lactate mediates XIAP protection of beta cells from autoimmunity, XIAP expression was induced in beta cells of 8-week old NOD mice via intra-pancreatic duct injection of AAV8-RIP-XIAP, with or without daily injection of the LDH inhibitor, oxamate. Mice were monitored for development of hyperglycemia. To assess whether beta-cell lactate production protects from diabetes in NOD mice, an AAV8 vector expressing LDH and MCT1 (AAV8-RIP-LDH-MCT1) was generated and injected intra-ductally into 8-week old NOD.scid mice.

Results:
XIAP expression in beta cells of NOD.scid mice increased expression of LDH and MCT1 without compromising glucose- or pyruvate-stimulated insulin secretion, despite increased lactate production. NOD mice with beta-cell expression of XIAP had markedly reduced incidence of diabetes compared to those who received XIAP plus oxamate or controls transduced with mock vector. Diabetes was associated with extensive insulitis and loss of beta cells. Six weeks following intra-ductal injection of AAV8-RIP-LDH-MCT1 into 8-week old NOD.scid mice, we found significant expression of LDH and MCT1, and increased glucose-stimulated lactate release in culture, compared to empty vector recipients. Female NOD mice transduced with these viruses and are being followed for development of diabetes.

Conclusions:
Our data suggest that one mechanism by which beta cell expression of XIAP may prevent autoimmune diabetes is via induction of genes normally repressed in beta cells, resulting in local islet production of the immunosuppressive metabolite lactate.

MARY KINLOCH

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

"Morphological Features Associated with POLE Mutations: Implications for Risk Assessment in Endometrial Carcinoma"

AUTHORS:
Mary Kinloch[1], Salwa Bakhsh[2], Rob Soslow[3], Martin Kobel[4], Cheng-Han Lee[5], Jessica McAlpine[6], Melissa McConechy[2], Blake Gilks[1]

AFFILIATIONS:
[1]Vancouver General Hospital, Department of Pathology and Laboratory Medicine; [2]BC Cancer Agency, Department of Pathology and Laboratory Medicine; [3]Memorial Sloan Kettering Cancer Center, Department of Pathology; [4]Calgary Laboratory Services, Department of Pathology and Laboratory Medicine; [5]Royal Alexandria Hospital, Department of Laboratory Medicine and Pathology; [6]Vancouver General Hospital, Department of Gynecologic Oncology

Background/objectives:
Endometrial cancer is the commonest gynecological cancer in developed countries Approximately 2.7% of women will be diagnosed with endometrial carcinoma (EC) in their lifetime. Since the identification of the four different genomic subsets of endometrial carcinoma by the Cancer Genome Atlas (TCGA), multiple studies have emerged in the literature trying to characterize morphologically a previously unrecognized group (ultramutated phenotype). This ultramutated genomic phenotype is associated with mutations in the exonuclease domain of polymerase epsilon (POLE) resulting in defective proofreading function during DNA replication.

Objective: The objective of this study was to identify the histomorphological parameters that would assist in diagnosing ECs harboring POLE mutations, to aid in risk assessment.

Methods:
A total of 500 cases of EC were assessed by Next Generation sequencing technology for POLE mutation and 47cases were identified (9.4%). Hysterectomy H&E slides were reviewed for 43 of the cases and evaluated for a range of histomorphologic criteria. Including; peritumoral lymphocytes, tumor intraepithelial lymphocytes (TIL), mitotic count per 10 hpf, nuclear grade, intratumoral heterogeneity, amoung others. Variables used for risk assessment and decision making about adjuvant therapy were also recorded, including FIGO stage, lymphovascular invasion (LVI), and FIGO grade. p53 mutation was assessed by IHC as well as the Mismatch Repair (MMR) status. The nuclear features and mitotic count of the POLE cases was compared to an unselected cohort of 209 EC cases from our institution (AAEC).

Results:
The majority of POLE mutated tumours are of endometroid type (~ 79%), but other histotypes such as serous and mixed carcinomas were also seen in the POLE mutated group. The median age of the patients was 59 years and the majority of the patients had early stage disease (FIGO I –II) (98%) with a single case of late stage (FIGO stage III). These tumors exhibited high grade features even though architectural features were typically low grade. Similar to MSI-H ECs these tumors were associated with presence of peritumoral lymphocytes (~79%), intratumoral lymphocytes (Range 19/10 HPF – 1398/10 HPF), and bizarre tumour giant cells. The mitotic count and high nuclear grade was statistically higher than the AAEC group ($p<0.005$). As per local treatment guidelines, ~ 41 % of patients would have been eligible in adjuvant chemotherapy regimen and ~ 79% in vault +/- pelvic radiotherapy.

Conclusions:
The morphology of POLE mutated tumors shows some differences but overlaps with that of other EC subtypes, therefore, diagnosis by routine H&E is not feasible. Additionally, current histopathological features used in decision making about the need for adjuvant treatment, when applied to these POLE mutated tumours, result in a significant number of patients receiving adjuvant therapy, even though POLE mutant ECs are associated with a very favorable prognosis.

KRIS MARTENS

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"CHIMERA (Closed Head Impact Model of Engineered Rotational Acceleration) Mild Traumatic Brain Injury Results in Cognitive Dysfunction in the APP/PS1 Transgenic Mouse"

AUTHORS:
Kris M. Martens[1], Wei Hang Cheng[1], Dhananjay R. Namjoshi[1], Peter Cripton[2], and Cheryl L. Wellington[1]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Departments of Mechanical Engineering and Orthopaedics, University of British Columbia

Background/objectives:
In addition to being a leading cause of disability in young people, traumatic brain injury (TBI) is a risk factor for dementia, including Alzheimer's disease (AD). We recently developed a novel rodent TBI model called CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) that uses a non-surgical procedure to precisely deliver defined impacts to an intact head with unrestrained and reliable head movement. In C57Bl/6, CHIMERA induces significant behavioral deficits. Here we apply CHIMERA TBI to the APP/PS1 model of AD.

Methods:
APP/PS1 transgenic mice, 6-months of age, received either two CHIMERA TBIs spaced 24h apart or sham procedure. Behavior was assessed up to 1-month post injury and was selected from the axes of sensory/motor (neurological severity score, rotarod), affective (elevated plus maze), and cognitive (Barnes maze, passive avoidance).

Results:
Mice subjected to CHIMERA repeated mild TBI showed no sensory or motor deficits post injury; yet, showed cognitive dysfunction on both the Barnes maze and passive avoidance compared to sham.

Conclusions:
These results support the hypothesis that CHIMERA TBI induces cognitive impairment in the APP/PS1 transgenic mouse. Future studies will reveal how CHIMERA TBI affects disease progression in APP/PS1 mice.

JANE O'HARA

Supervisor: Dr. Niamh Kelly
Session: Basic Sciences

"Creative Science: Effects of Engaging with Creativity on High School Students Attitudes and Beliefs About Science"

AUTHORS:
Jane E. O'Hara[1], Deborah Butler[2], Niamh Kelly[1]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine; [2]Department of Educational and Counselling Psychology and Special Education, University of British Columbia

Background/objectives:
A level of interest and understanding of science is arguably essential for adults to make informed decisions about many issues including those related to health and the environment. At the high school level, engagement in science has been decreasing steadily over time. Recent discussion has focused on the need for educational approaches that motivate high school students not only to continue studying science, but to appreciate the relevance and importance of science in daily life and the world around them. The aim of this study was to investigate whether engagement in learning science through creative expression would affect high school students' attitudes and beliefs about science and about themselves as learners of science.

Methods:
High school classes ranging from grade 8 to 12 were recruited to take part in this study. After parental consent was obtained, students (n=58) were asked to complete a 30-question survey designed to collect information about their attitudes and beliefs about science. One week later, they began a project called 'Creative Science', whereby students represent their learning of a particular topic in the school science curriculum by generating an artistic or creative product. Time given to complete the project was 6-10 weeks. One week after submission of the projects, students were asked to complete the same 30-question survey as before. Qualitative and quantitative data were obtained from the surveys and used to evaluate attitudinal shifts in the cohorts overall, as well as in individual students. Attitudes of grade 8 students were also compared with those of grade 12.

Results:
From the pre-project survey data, two groups emerged: one comprised of students who tended to describe science as relating to laboratories, experiments, chemicals and other such concrete items, and the other comprised of those who described science as a way of looking at and understanding the world and/or a creative and imaginative enterprise. Within the first group, students were identified as having attitudes about science that were either overarchingly positive or negative (measured by self-reporting about enjoyment of and interest in science). In the second group, almost all students could be identified as having an overarching positive attitude about science. We compared these responses to the same questions in the post-project survey from the group as a whole, and found that overall attitudes about the relevance of science had shifted to the positive side (relevance of science to understanding and solving world problems, and relevance of science to the student's personal world). Additionally, when we examined the responses of individual students whose pre-project attitudes to science were the most negative, some positive shifts had occurred. Students whose attitudes were positive in the beginning tended to remain positive about science after the completion of the Creative Science project.

Conclusions:
These early results point to the fact that the intentional introduction of creativity into the science education of high school students can have a positive effect on students who have negative attitudes about science and their ability to learn it. This study will be expanded in the 2015-2016 academic year with new schools and classes joining the Creative Science program, ensuring a larger study cohort.

JÉRÔME ROBERT

Supervisor: Dr. Cheryl Wellington
Session: Basic Sciences

"Development of a Novel Tissue Engineered Model of the Cerebrovasculature"

AUTHORS:
Jérôme Robert, Sophie Stukas, Tammy Wilson, Cheryl L Wellington

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

Background/objectives:
The complex interrelationship between cerebrovascular dysfunction and Alzheimer's Disease (AD) is increasingly appreciated. Indeed 30% of AD patients have cerebral infarcts at autopsy, and up to 90% demonstrate evidence of cerebral small vessel dysfunction including deposition of amyloid beta (Ab) within the vascular wall. This Ab deposition is associated with cerebrovascular and blood-brain barrier (BBB) dysfunction. Compared to the wealth of knowledge about the vascular physiology of large peripheral vessels, little is known about the mechanisms by which vascular risk factors for AD lead to cerebral small vessel dysfunction. The potential magnitude of this knowledge gap is put into perspective when one considers that every neuron is partnered with its own capillary. The need to better understand cerebral small vessel function and dysfunction in dementia appears therefore of particular interest.

Methods and Results::
We recently developed a unique human based tissue engineering approach to develop a 3D functional model of the cerebrovasculature, which is composed of either vascular smooth muscle (SMC) and endothelial cells (EC) or astrocytes, SMC and EC culture under flow conditions in vitro. Anteluminal injection of Ab42 in the engineered vessel induces time and dose dependent accumulation of amyloid within the tissue and a subsequent activation of the EC measured by adherence of circulating monocytes.

Conclusions:
In conclusion, the described tissue engineered human vascular equivalent model represents a significant step towards a relevant in vitro platform for the systematic assessment of pathogenic processes in AD independently of any systemic factors.

STEVE SHEN

Supervisor: Dr. David Granville
Session: Basic Sciences

"Granzyme B Deficiency Protects Against Angiotensin II-induced Cardiac Fibrosis via a Perforin-Independent Mechanism"

Background/objectives:
Cardiac fibrosis is a common pathological feature of many heart diseases, and a hallmark feature of chronic heart failure. Heart failure affects more than five million people in North America, and costs over $32 billion annually in health care services, medications and lost productivity. Approximately half of the patients diagnosed with heart failure die within 5 years, often as a direct consequence of reduced cardiac function. Granzyme B (GzmB) is a serine protease with multifunctional roles. In the old dogma, GzmB is believed to be released exclusively along with the pore-forming protein perforin to induce apoptotic cell death. Over recent years, this dogma has been challenged, given the large amount of data showing that GzmB accumulates in the extracellular space of inflamed tissue, and is present and retains its activity in body fluids collected from patients with diseases associated with aging and chronic inflammation. Furthermore, it has been shown that GzmB has a profound perforin-independent role in the pathogenesis of a number of chronic inflammatory and age-related cardiovascular diseases. However, there is no direct link yet established between GzmB and cardiac fibrosis. In the present study we investigated the role of GzmB in the pathogenesis of cardiac fibrosis.

Methods:
Using a murine model of Angiotensin II (Ang II)-induced cardiac fibrosis, wild-type, GzmB deficient and Perforin deficient mice were treated with Ang II for 4 weeks, and were examined for the presence of cardiac fibrosis.

Results:
Our results showed that GzmB is up-regulated in both human and mouse fibrotic hearts. Genetic deficiency of GzmB notably protects against Ang II-induced cardiac dysfunction, hypertrophy and fibrosis, independently of perforin. GzmB directly cleaves VE-cadherin, a key endothelial cell-cell junction protein, and contributes to the disruption of endothelial barrier function and increased vascular permeability. GzmB deficiency attenuates cardiovascular permeability, cardiac inflammation and fibroblast accumulation in Ang II-infused hearts, and eventually inhibits the pathogenesis of cardiac fibrosis.

Conclusions:
Our results indicate a perforin-independent, extracellular role for GzmB in the pathogenesis of cardiac fibrosis, and suggest that targeting extracellular GzmB should be considered as a potential strategy to intervene on the progression of cardiac fibrosis.

AUTHORS:
Steven Yue Shen[1,2], Fang Cheng[3], Mehul Sharma,1 Yulia Merkulova[1], Sheetal A. Raithatha[1,2], Leigh G. Parkinson[1,2], Hongyan Zhao[1,2], Kathryn Westendorf[1,2], Lubos Bohunek[1,2], Tatjana Bozin[1,2], Ivy Hsu[1], Lisa S. Ang[1], Sarah J. Williams[1,2], R Chris. Bleackley[4], John E. Eriksson[3], Michael A. Seidman[1,2], Bruce M. McManus[1,2], and David J. Granville[1,2]

AFFILIATIONS:
[1]Centre for Heart Lung Innovation, St. Paul's Hospital; [2]Department of Pathology and Laboratory Medicine, University of British Columbia; [3]Department of Biosciences, Åbo Akademi University and Turku Centre for Biotechnology, Turku, Finland; and [4]Department of Biochemistry, University of Alberta

CHEN WANG

Supervisor: Dr. Honglin Luo
Session: Basic Sciences

"Dysferlin Deficiency Confers Increased Susceptibility to Coxsackievirus-Induced Cardiomyopathy"

AUTHORS:
Chen Wang

AFFILIATIONS:
[1]James Hogg Research Center, Providence Heart + Lung Institute, St. Paul's Hospital and Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Background/objectives:
Coxsackievirus infection can lead to viral myocarditis and its sequela, dilated cardiomyopathy, which represent major causes of cardiovascular mortality worldwide, particularly in children. Yet, the host genetic susceptible factors and the underlying mechanisms by which viral infection damages cardiac dysfunction remain to be fully resolved. Dysferlin is a transmembrane protein highly expressed in skeletal and cardiac muscles. In humans, mutations in the dysferlin gene can cause limb-girdle muscular dystrophy type 2B and Miyoshi myopathy. Dysferlin-deficiency is also associated with and is linked to cardiomyopathy. Defects in muscle membrane repair have been suggested to be an important mechanism responsible for muscle degeneration in dysferlin-deficient patients and animals.

Methods:
For in vivo experiment, we use Male A/J (n=10), C57BL/6 (n=10), 129S1/SvlmJ (n=16), and dysferlin-null (129-Dysftm1kcam/J, n=16) mice. Infected intraperitoneally with CVB3 for 7 or 9 days and Infected heart was harvested for western blot analysis, histological examination, in situ hybridization, and plaque assay. For in vitro experiment, we use HL-1 cell line, which is established from a mouse atrial cardiomyocyte tumor lineage and Hela cells. They were infected with CVB3 at a multiplicity of infection (MOI) of 50 and 10, respectively, or sham-infected with phosphate-buffered saline (PBS). After 1 hour (h) infection, the medium was washed with PBS, and replaced with refresh medium. For cDNA overexpression, HeLa cells were transiently transfected with plasmids using Lipofectamine® 2000. Western blot was used to detect expression of dysferlin and its cleavage.

Results:
In this study, we demonstrated that loss of dysferlin confers increased susceptibility to coxsackievirus infection and myocardial damage. We also found that dysferlin is cleaved following coxsackieviral infection through the proteolytic activity of virally encoded protease 2A.

Conclusions:
Our results in this study not only identify dysferlin-deficiency as a novel host risk factor for viral myocarditis but also reveal an important mechanism by which coxsackievirus infection impairs cardiac function, leading to the development of dilated cardiomyopathy.

GARRETT BARRY

Supervisor: Dr. Randy Gascoyne
Session: Clinical Sciences

"Effect of Tumour Content on Lymph2Cx Cell-of-Origin Classification of Diffuse Large B-cell Lymphoma by in Silico Dilution with Reactive Lymph Node"

AUTHORS:
Garrett Barry[1], Anja Mottok[1], Daisuke Ennishi[1], David Scott1, Randy Gascoyne[1]

AFFILIATIONS:
[1]Centre for Lymphoid Cancer, BC Cancer Research Centre, Vancouver, BC

Background/objectives:
Diffuse large B-cell lymphoma (DLBCL) is an aggressive hematologic malignancy originating from maturing B-cells within lymph nodes or extranodal tissues. The "Lymph2Cx" 20-gene expression-based classifier separates DLBCL tumours into two prognostically significant cell-of-origin subtypes: germinal-centre B-cell-like (GCB) and activated B-cell-like (ABC). Minimal tumour content cutpoints for the classifier assay tissue requirements has been previously set at 60%. The objective of this project was to estimate a new tumour content cutpoint in silico using data from a large Lymph2Cx study with the goal of optimizing the number of DLBCL tumours that can be reliably assessed by Lymph2Cx.

Methods:
Lymph2Cx Nanostring nCounter assays were performed using 200ng of RNA extracted from scrolls of formalin-fixed paraffin embedded tissue (FFPET) from 344 de novo DLBCLs treated with R-CHOP and 5 reactive lymph nodes (RLN), as per standard protocols. Normalized Lymph2Cx data was used to perform an in silico dilution of each DLBCL case with ≥90% tumour content with each RLN case in duplicate at each 10% tumour content interval from 0% to 100%. Lymph2Cx algorithm scores were used to determine the likelihood of a case being an ABC DLBCL and calculate misclassification rates at each dilution. Kaplan-Meier curves were drawn to compare the time-to-progression and disease-specific survival between Lymph2Cx-defined subtypes using a ≥40% tumour content cutpoint for inclusion.

Results:
Of 344 DLBCLs, 107 ABC or GCB cases (43/107 ABC) had a tumour content ≥90% and were used in the in silico dilution experiment. RLNs were classified as either GCB or were unclassifiable by Lymph2Cx. Overall, ABC-type DLBCLs gradually shifted towards a GCB-type classification with increasing dilution with the RLN data. Misclassification rates of true ABCs as GCBs were 5.35%, 11.6%, and 16.7% at 50%, 40%, and 30% tumour content cutpoints, respectively, which suggested that a 40% tumour content cutpoint may be a rational cutpoint. This was further supported by the cohort distribution of true ABC cases to true GCB and unclassifiable cases remaining constant down to 20% tumour content. Using the 40% tumour content cutpoint for inclusion, ABCs conferred a significant risk of progression (TTP; HR=2.5, log rank $P < 0.0001$) and inferior survival (DSS; HR=2.6, log rank $P < 0.0001$) compared to the GCBs.

Conclusions:
Previously, only DLBCL tumours containing ≥60% tumour content were included in Lymph2Cx gene expression studies, comprising approximately 80% of DLBCLs. This in silico dilution experiment suggests that a cutpoint of ≥40% remains reliable with a low misclassification rate (11.6%) and high prognostic significance between DLBCL subtypes. Approximately 90% of DLBCL cases are candidates for the Lymph2Cx using this experimentally-derived tumour content cutpoint.

PAWAN DHALIWAL

Supervisor: Dr. Mari DeMarco
Session: Clinical Sciences

"Investigation of Aberrant ACTH Isoform as a Result of a Suspected Silent Corticotroph Adenoma"

AUTHORS:
Pawan Dhaliwal[1], Emma Zheng[1], Mari DeMarco[1,2]

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

Background/objectives:
A 64-year-old male ("patient") with a silent corticotroph adenoma producing a suspected non-functional isoform of adrenocorticotropic hormone (ACTH) is presented. Laboratory investigations revealed consistently elevated plasma ACTH concentrations, however cortisol levels were within reference range and illustrated normal diurnal variation. The patient did not present with signs and symptoms of elevated ACTH, such as skin hyperpigmentation, weight loss, joint or muscle pain. A CT scan of the head/neck revealed the presence of a 23 mm multi-lobulated lesion in the left parasellar region. Immunohistochemical studies on a resected portion of the tumor demonstrated immunopositivity of tumor cells for ACTH. To characterize the isoform(s) of ACTH present in patient plasma, we developed an immunoprecipitation—liquid chromatograph—mass spectrometric method.

Methods:
The ACTH plasma concentration of all plasma specimens was assessed on the Siemens Immulite. In addition, the ACTH concentration of the patient was assessed on the Roche instrument to assess the ability of the Roche antibodies to cross-react with the aberrant ACTH isoform. ACTH in patient and control plasma was immunoprecipitated using antibody-bound magnetic particles. The eluate was dried via vacuum centrifugation and reconstituted with 10 microliters of sample buffer for liquid chromatography-tandem mass spectrometric (LC-MS/MS) analysis on a SCIEX 5500 QTRAP. Mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). A precursor ion scan was performed from mass-to-charge ratio of 100 to 1250. Product ion scans were performed at a declustering potential of 100 V, with collision energy of 40 V.

Results:
Consistent with the findings from the Siemens assay, the Roche antibodies also cross-reacted with the aberrant ACTH isoform yielding an elevated ACTH concentration. Immunoprecipitation efficiency of the magnetic bead protocol was approximately 74 ± 8%. Mass spectrometric analysis confirmed the presence of wild-type ACTH in both patient and control samples. Further differential analysis of the precursor ion spectra of patient and control samples revealed an abundant peak in the patient specimen consistent with presence of an N-terminally truncated form of ACTH.

Conclusions:
We successfully developed an immunoprecipitation protocol to purify femtomole quantities of ACTH from human plasma. Via mass spectrometric analysis we confirmed the identity of wild-type ACTH (residues 1-39) in both patient and control samples. Preliminary evidence indicates that the patient sample contains a significantly elevated amount, relative to control plasma, of an N-terminally truncated ACTH isoform (residues 3-39). As residues 1-24 of ACTH are required for biological activity, the presence of this isoform is consistent with the clinical picture and laboratory findings. Further experiments are underway to fully characterize and quantitate the mis-processed ACTH isoform.

SANDRA BOTROS

Supervisor: Dr. John Priatel
Session: Basic Sciences

"Correcting a Primary Immunodeficiency Disease Using CRISPR-Cas9 Genome Editing"

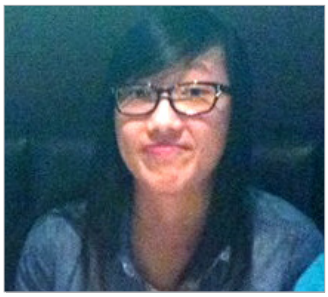
AUTHORS:
Sandra Botros[1], Kevin Tsai[1], Francis C Lynn[1], and John J Priatel1

AFFILIATIONS:
[1]Child and Family Research Institute, University of British Columbia, Vancouver BC

Background/objectives:
Primary immunodeficiencies are genetic disorders in which part of the body's immune system is missing or does not function properly with affected individuals being especially prone to infections and inflammatory diseases. Current therapeutic regimens for severe diseases involve hematopoietic stem cell transplantation through bone marrow transplant, but this carries serious risks such as life-threatening infections and graft-versus-host diseases. Recent studies suggest the potential of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) method of genetic recombination to correct genetic defects in the mammalian genome. The CRISPRCas9 system utilizes a designed guide RNA to engineer DNA breaks at a specifically defined locus, and provision of an oligonucleotide template mediates homology-directed repair of the mutated gene.

Methods:
Primary immunodeficiencies are genetic disorders in which part of the body's immune system is missing or does not function properly with affected individuals being especially prone to infections and inflammatory diseases. Current therapeutic regimens for severe diseases involve hematopoietic stem cell transplantation through bone marrow transplant, but this carries serious risks such as life-threatening infections and graft-versus-host diseases. Recent studies suggest the potential of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) method of genetic recombination to correct genetic defects in the mammalian genome. The CRISPRCas9 system utilizes a designed guide RNA to engineer DNA breaks at a specifically defined locus, and provision of an oligonucleotide template mediates homology-directed repair of the mutated gene.

Projected Results::
We expect *Scurfy* cells assessed after transfection with CRISPR-Cas9 to show increased CD25+FOXP3 C-terminal+ fluorescence compared to untreated cells via Flow Cytometry. We also expect injection of the CRISPR-Cas9 -repaired T cells into *Scurfy* mice, allowing them to take on the role of functional T-reg cells, will thereby provide a functional cure.



CELINE CHAN

Supervisor: Dr. Chun Seow
Session: Basic Sciences

"Mechanical Manifestation of Smooth Muscle Myosin Light Chain Phosphorylation"

AUTHORS:
Celine Chan[1], Saleha Banaem[1,2], Lu Wang [2], Peter D. Paré[2], Chun Y. Seow[1,2]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Centre for Heart Lung Innovation

Background/objectives:
It is well known that phosphorylation of the myosin light chain (MLC) at the serine-19 residue by myosin light chain kinase initiates contraction in smooth muscle. However, the role of MLC phosphorylation in the regulation of cross-bridge kinetics is still poorly understood. If MLC phosphorylation acts as a switch to turn on the cross-bridges, the level of MLC phosphorylation should correlate with the number of activated bridges and hence the power output of the muscle. Our goal is to determine the correlation between MLC phosphorylation and the force-velocity properties of smooth muscle including the maximum shortening velocity (Vmax) and the maximum power output (Pmax).

Hypothesis: MLC phosphorylation activates smooth muscle cross-bridges and the level of phosphorylation correlates with the maximum power output of the muscle.

Methods:
We performed two sets of parallel experiments on sheep tracheal smooth muscle strips. In the first set of experiments, we examined the effect of the protein kinase C (PKC) inhibitor GF109203x (6 μM) on the mechanical properties of airway smooth muscle. We obtained force-velocity curves by performing isotonic quick releases where the muscle was allowed to shorten against various loads after activation. We then determined maximum shortening velocity and power (Vmax, Pmax) from the force-velocity curves. In the second set of experiments, we examined the effect of the PKC inhibitor on MLC phosphorylation levels using Western blots on muscle strips frozen at the plateau of force generation. We then quantified MLC phosphorylation by densitometry analysis.

Results:
Vmax decreased 2.56% ± 2.31% and 18.88% ± 1.95% for the control and the test (PKC inhibitor) respectively after an hour of incubation ($p < 0.001$) whereas Pmax decreased 16.71% ± 2.93% and 40.44% ± 6.61% for the control and the test ($p = 0.011$). We found no difference in the levels of MLC phosphorylation between the control and the test ($p = 0.056$; n=3).

Conclusions:
Although the PKC inhibitor induced a significant decrease in maximum shortening velocity (Vmax) and maximum power output (Pmax) in tracheal smooth muscle, our preliminary results indicate that neither Vmax nor Pmax correlated with MLC phosphorylation.

BRIAN CHO

Supervisor: Dr. Decheng Yang
Session: Basic Sciences

"Coxsackievirus B3 Induces Expression of Heat Shock Protein 70 to Favour its Replication"

AUTHORS:
Brian Y. Cho[1,2], Ye Qiu[2], Xin Ye[2], Jeff Zong[2], Mary Zhang[2], Paul J. Hanson[2], Decheng Yang[2]

AFFILIATIONS:
[1]MD Undergraduate Program, Faculty of Medicine, University of British Columbia; [2]Centre for Heart Lung Innovation, UBC and St. Paul's Hospital, Department of Pathology and Laboratory Medicine, University of British Columbia

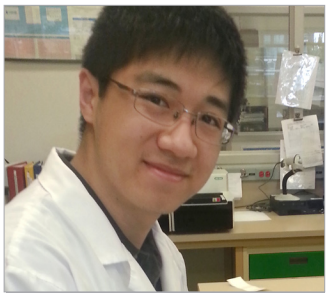
Background/objectives:
Coxsackievirus B3 (CVB3) is a major pathogen of viral myocarditis, a leading cause of sudden death in young people, and can progress to dilated cardiomyopathy. The CVB3 genome is a positive, single-stranded RNA that is translated via an internal ribosome entry site (IRES)-mediated mechanism as opposed to cap-dependent translation of most cellular mRNAs. Upon infection, CVB3 induces cellular stress that shuts down global cap-dependent translation and promotes IRES-mediated translation. It was previously determined that heat shock 70 kDa protein 1A (Hsp70), a cellular chaperone protein, is up-regulated in cellular stress such as viral infections. In fact, the mRNA of Hsp70 has an IRES site, which potentially allows it to be translated during CVB3 infection. Our aim is to determine the mechanism by which CVB3 up-regulates Hsp70 and to determine the feedback effect of Hsp70 on CVB3 replication.

Hypothesis: CVB3 will up-regulate Hsp70 through phosphorylation of heat shock factor 1 (HSF-1), a transcription factor for Hsp70. Then, Hsp70 will positively feedback on CVB3 replication by stabilizing the viral genome.

Methods:
Hsp70 up-regulation: HL-1 cardiomyocytes were infected with either sham or CVB3. At 6~10h post infection, Hsp70 protein and mRNA were detected by Western blot and quantitative real-time PCR, respectively. Role of CaMKII and HSF-1: HeLa cells were infected with sham or CVB3 for 2~6h. Phosphorylated CaMKII and phosphorylated HSF-1 (at Ser230) were detected by Western blot. Nuclear extraction: HeLa cells were infected with CVB3 for 3~6h. Nuclear and cytoplasmic proteins were isolated from cell lysates and analyzed by Western blot using HSF-1 antibodies. CVB3 genome stabilization: HeLa cells were transfected with Hsp70-siRNA to knockdown Hsp70, or transfected with pEGFP-Hsp70 to overexpress Hsp70. After infection with CVB3 for 2~4h, the cells were analyzed by qRT-PCR to detect RNA levels of 2A gene (CVB3 genome indicator). The role of the CVB3 ARE site, which is critical for RNA turnover, was determined by dual luciferase assay.

Results:
Hsp70 up-regulation: HL-1 cardiomyocytes showed increased expression of Hsp70 by 6 hours post infection (hpi). Role of CaMKII and HSF-1: CaMKII was phosphorylated starting at 3 hpi, followed by phosphorylation of HSF-1 at 4 hpi. Nuclear extraction: Nuclear extraction results demonstrated that HSF-1 translocated from the cytoplasm to the nucleus starting at 4 hpi. CVB3 genome stabilization: Cells with Hsp70 knockdown by Hsp70-siRNA had a decrease in CVB3 genomic RNA, while cells with overexpressed Hsp70 (pEGFP-Hsp70) had an increase in the viral genomic RNA.

Conclusions:
CVB3 exploits the host cell's machinery for its replication by inducing the expression of Hsp70. Hsp70 is up-regulated through pCaMKII-mediated phosphorylation of HSF-1 at Ser230, with pHSF-1 acting as a transcription factor for Hsp70 mRNA synthesis. Subsequently, Hsp70 positively feeds back on CVB3 replication by stabilizing the viral genome.



CHARLIE HUA

Supervisor: Dr. Mari L. DeMarco
Session: Clinical Sciences

"Investigation of a Mass Spectrometric Approach for Detection of α-synuclein in Neurodegenerative Disease"

AUTHORS:
Charlie Hua[1], Grace van der Gugten[2], Mari L. DeMarco[1,2]

AFFILIATIONS:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Department of Pathology and Laboratory Medicine, St. Paul's Hospital, Providence Health Care

Background/objectives:
The protein alpha-synuclein (α-syn) is the primary component of the pathogenic inclusions known as Lewy bodies. Lewy bodies are associated with many neurodegenerative diseases including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Current efforts to characterize α-syn as a biomarker in body fluids, such as cerebrospinal fluid (CSF), rely on immunometric technologies. However, cross-reactivity and poor-reproducibility of such methods has limited their utility in characterizing α-syn as a disease biomarker. Given these obstacles, we chose to take an alternate approach and develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of α-syn in CSF.

Methods:
Method development included: in silico protein/tryptic peptide characterization of α-syn, LC-MS/MS method development, and assessment of time-course peptide response, digestion matrices and analytical sensitivity. To begin we used in silico digests, structural characterization and proteomic database searching to identify candidate α-syn tryptic peptides. We then tested preliminary digest procedures and observed tryptic peptides by LC-MS/MS. For this step, recombinant human α-syn was digested with trypsin at a 1:20 or 1:50 α-syn-to-trypsin ratio in a 37°C thermoshaker for up to 32 hours. The α-syn precursor and product ions were detected on an SCIEX 5500 QTRAP instrument. We then characterized peptide responses as a result of digestion in various matrices: ammonium bicarbonate buffer, artificial CSF (contains no proteins), artificial CSF spiked with 0.4% plasma, and CSF. Finally, the detection limit was determined through serial dilutions of a known concentration of α-syn followed by digestion and detection by LC-MS/MS.

Results:
Six candidate peptides with the following masses were detected following trypsin digestion and LC-MS/MS analysis: 830.92, 1296.47, 1479.67, 1607.85, 1929.18, and 2158.46 Da. The peptide response curves generated from the duration of digestion indicated that detection signal plateaus between 16 and 20 hours. The 1:50 trypsin ratio yielded similar performance compared to the 1:20 ratio. Of these six identified candidate peptides, one peptide (1296.47) showed a decrease in response in the artificial CSF spiked with plasma and CSF matrices, likely due to endogenous proteases. All other peptides displayed typical digestion profiles. A preliminary assessment of the detection limit showed that this workflow was able to reliably detect α-syn down to 50 ng/mL.

Conclusions:
An initial workflow of a 16 hour digestion at an 1:50 α-syn-to-trypsin ratio has been established here. This current method provides sensitive detection in biological matrices in the ng/mL range.

ERIKA KOECK

Supervisor: Dr. Patrick Doyle
Session: Clinical Sciences

"Xpert MTB/RIF as a Supplemental Test to Acid-fast Bacilli Sputum Microscopy in the Diagnosis of Tuberculosis: Potential Resource Savings"

AUTHORS:
Erika Koeck[1], Titus Wong[1,2], Diane Roscoe[1,2], Patrick Doyle[1,2]

AFFILIATIONS:
[1]University of British Columbia; [2]Vancouver General Hospital

Background/objectives:
Diagnosis of pulmonary tuberculosis remains a challenge due to the low sensitivity of sputum microscopy and long culture turn-around times. Xpert MTB/RIF (Cepheid, Sunnyvale, CA) is a cartridge-based PCR assay which provides a higher sensitivity than microscopy and has exceptional turnaround time and ease-of-use. These advantages come with an increased per test cost. However, the increased cost of using Xpert MTB/RIF as a supplemental test to sputum microscopy may be offset by savings produced by better case detection.

Methods:
In this pilot study, retrospective data from1 large academic tertiary care centre and 2 community hospitals was fit into a theoretical model to determine whether routine implementation of Xpert MTB/RIF impacts resource utilization. A detailed cost-analysis was not part of this study. Data from a review of the microbiology specimen databases for the period January 1 2012 to December 31 2013 was used to determine whether airborne isolation time, infection control practitioner workload, and number of bronchoscopies performed would be impacted. Resource savings were calculated based on performing a single Xpert MTB/RIF assay per case, on the first sputum produced.

Results:
It was estimated that 36.2 (95% CrI 36.0 - 36.6) days of airborne isolation, 24.5 (95% CrI 21.2 - 27.0) hours of infection control practitioner time and 12.7 (95% CrI 12.4-13.0) bronchoscopies could have potentially been avoided had Xpert MTB/RIF been performed on the first sputum in the 534 cases that were included in this study. The additional cost for 534 Xpert MTB/RIF cartridges at $49.98 per test totaled $26 689, exclusive of technologist time. Savings for bronchoscopies that were avoided were calculated using $1946 per uncomplicated bronchoscopy for a savings of $25 298 in bronchoscopy fees.

Conclusions:
It appears from the results of this study that bronchoscopy savings alone are nearly enough to justify Xpert MTB/RIF implementation. These initial results suggest that further investigation is warranted with respect to the cost-effectiveness of routinely implementing Xpert MTB/RIF in clinical microbiology laboratories serving comparable hospitals in Canada.

ARMAAN MALHOTRA

Supervisor: Dr. Marcel Bally
Session: Basic Sciences

"Optimization of Carboplatin Liposome Formulation for Application to Stage IV Astrocytoma Therapy"

AUTHORS:
Armaan Malhotra[1], Mohamed Wehbe[1,3], Malathi Anantha[1] and Marcel Bally[1,2,3]

AFFILIATIONS:
[1]Experimental Therapeutics, British Columbia Cancer Agency Cancer Research Centre; [2]Department of Pathology and Laboratory Medicine, University of British Columbia; [3]Pharmaceutical Sciences, University of British Columbia

Background/objectives:
Glioblastoma Multiforme (GBM) is the most common and aggressive primary central nervous system neoplasm; it is associated with a median survival time of 12-14 months with current therapeutic interventions applied. The standard of care for GBM – as with many other brain cancers - has not changed in the past decade; it combines surgical resection, radiotherapy and chemotherapy. GBM drug delivery efforts are continuously stifled by the blood brain barrier (BBB), which is selective to lipophilic molecules and expresses efflux transporters on the surface. Carboplatin is a slightly lipophilic platinum based DNA alkylating chemotherapeutic that has exhibited cytotoxicity to glioma cells. The research summarized here describes the development of carboplatin-encapsulated lipid nanoparticles (LNPs) for intended use in GBM therapy. LNPs are phospholipid bilayers comprised of selected saturated fatty acids surrounding an aqueous solution. This project hypothesizes that for chemotherapeutics with a natural propensity to cross the BBB, an increase in circulation time through re-formulation in LNPs will improve drug delivery to the brain.

Methods:
The formulations created throughout this study utilized combinations of 1,2-distearoyl-sn-glycero-3-phosphoglycerol (DSPG), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol (CHOL). Lipids are initially dissolved in an organic solvent (along with tritium-labelled choleteryl ester) and rehydrated using sucrose-HEPES buffer. Using high-pressure extrusion, the population of multilamellar vesicles is then reduced to unilamellar vesicles at a predictable size (100 nm diameter). Carboplatin encapsulation was accomplished using passive equilibration, which involves immersing the LNP's in carboplatin-saturated buffer and elevating the temperature above the phospholipid phase transition temperature. Drug-to-lipid ratios were tracked using atomic absorption spectroscopy and liquid scintillation counting.

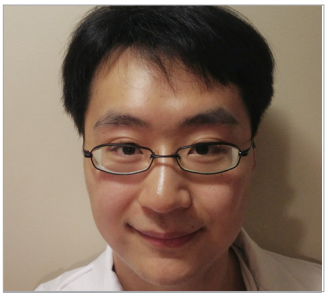
Results:
The first stage in the development of a LNP drug formulation for use as a chemotherapeutic agent involves finding a suitable liposome lipid composition, buffer and drug equilibration conditions for optimal loading. Both DSPC:Chol:DSPG (70:10:20 ratio) and DSPC:Chol (55:45 ratio) formulations were developed and found to exhibit reliable loading, stability and size. Carboplatin-loading capacity was greatly augmented by the use of ethanol during the passive equilibration process, which led to a more than two-fold improvement in encapsulation efficiency. Both loaded formulations were stable for 30 days at 4 degrees Celsius. Cytotoxicity was demonstrated *in vitro* and size fluctuations were insubstantial over the course of loading, storage and at in simulated *in vivo* conditions at 37 degrees Celsius.

Conclusions:
Two carboplatin LNP formulations were developed. The loading technique was optimized for encapsulation efficiency through the use of ethanol during the passive equilibration procedure. Drug to lipid ratios larger than those reported in current literature were achieved. This study should be followed by in vivo maximum tolerated dose and detailed pharmacokinetic investigations into the therapeutic potential of the formulation.

YUEYANG SHEN

Supervisor: Dr. Maria Monsalve
Session: Basic Sciences

"Mineral Inclusions in a Corpse Frozen Long Ago in a Canadian Glacier: X-ray Spectrometry of the Lung Tissue"



AUTHORS:
Yueyang Shen[1], Elaine Humphrey[2], David Walker[1], Maria Victoria Monsalve[1]

AFFILIATIONS:
Department of Pathology and Laboratory Medicine, University of British Columbia; Advanced Microscopy Facility, University of Victoria

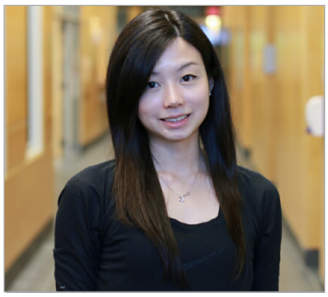
Background/objectives:
In August 1999, hunters found the frozen remains of an approximately 18-year-old male melting out of a glacier in the northwest corner of British Columbia.. His clothing and bone collagen were carbon dated 150-300 years old.

Raman spectroscopy previously performed on the lung tissue had indicated the presence of: l) order carbonaceous (graphene and graphite); 2) disordered carbonaceous (soot); 3) minerals (likely conglomerates) and 4) unidentified material (likely of organic origin, possible a contaminant). Here, our objective was to use scanning electron microscopy in combined with X-ray spectroscopy and elemental mapping to identify chemical composition of the minerals found in the lung tissue of the remains.

Methods:
Tissue was kept at -80oC before processing. Standard precautions minimized the risk of contamination. The lung tissue was processed by freeze substitution. Leica Automatic Freeze Substitution unit was used. The tissue was first fixed in acetone with 1% glutaraldehyde and 0.1% UA at - 85oC and post fixed in osmium tetroxide ramping up the temperature to - 20oC. Spurr-Epon resin mix was infiltrated at room temperature using the Pelco 3450 Laboratory Microwave. The resin was polymerized at 60oC overnight. The X-ray spectroscopy and elemental mapping were performed using Hitachi S-4800 field emission scanning electron microscope at the Advanced Microscopy Facility, University of Victoria. We measured “characteristic x-ray” emitted by the sample suing energy dispersive x-ray spectrometer.

Results:
X-ray spectrometry and elemental mapping indicated: 1) presence of carbon, oxygen, magnesium, silicon, aluminum, sodium, gold, lead, boron and iron; 2) inclusions of carbon particles, calcium, iron, gold, and magnesium; 3) fluorine, and silicon in the superficial tissue.

Conclusions:
We were able to determine elements that are part of the composition of inclusions. Presence of elements such as carbon, and oxygen was expected as they are part of the normal composition of a human body. Silicon and aluminum were found but not embedded in the tissue and may indicate contamination. The presence of gold and lead may suggest particles inhaled to his lungs.



JENNY WU

Supervisor: Dr. David F. Schaeffer
Session: Basic Sciences

"Assessment of Tumour Budding as a Determinant of Long-term Survival in Pancreatic Ductal Adenocarcinoma"

AUTHORS:
Jenny Wu, Kate O'Connor, Steve E. Kalloger, Joanna Karasinska, Daniel J. Renouf, David F. Schaeffer

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

Background/objectives:
Pancreatic ductal adenocarcinoma (PDAC) is the 4th most common cause of cancer related death in Canada. The 5-year overall survival rate for PDAC patients is a dismal 5%, although the rates are higher (10-20%) for the subset of patients who present with resectable tumour. The development of clinically relevant biomarkers that predict outcome and treatment response as well as novel therapies targeting the unique PDAC microenvironment represent a critical unmet need in this disease. The role of epithelial-to-mesenchymal transition (EMT) has been implicated as a mechanism of tumour invasiveness in PDAC and other cancers. Recently, we discovered that a putative histologic correlate of EMT, referred to as tumour budding (Tb), is significantly associated with poor prognosis in PDAC. Tb is defined by the presence of single malignant cells or small cell clusters (less than 5 cells) at the tumour invasive front. We found that 80% of PDAC patients undergoing surgical resection exhibit Tb and that budding was associated with lymphovascular invasion (p=0.0047) and inferior overall survival (p=0.001). The goal of this study is to determine if the presence of Tb can be used to define a clinically valid cut-point to classify PDAC patients as long-term (LTS) and short-term (STS) survivors.

Methods:
Resected PDAC cases from the Vancouver Coastal Health Region between 1987 – 2013 were selected for this study. Full slide review was performed to assess for the presence of Tb. Clinical follow-up and outcome data were derived from the BC Cancer Agency. Disease specific survival for the entire cohort was modeled with the Kaplan-Meier method. Tumour budding specific recursive partitioning was employed to determine the optimal LTS / STS cut-point utilizing an a priori decision to use the first DSS cut-point greater than the median disease specific survival time of the entire cohort. Differential prevalence of Tb between the LTS & STS groups was assessed with Fisher's Exact Test. Statistical independence of tumour budding prevalence amongst other conventional clinico-pathologic parameters in determining LTS from STS was determined with nominal logistic regression.

Results:
Tumour budding was assessed in 260 and was present in 230 (88.5%) PDAC patients. Median survival time for the entire cohort was estimated at 1.49 [1.33 – 1.73] years. Recursive partitioning analysis yielded one split that was incompatible with our a priori criteria at 0.56 years. The second split yielded a cut-point of 3.90 years. Using 3.90 years as the cut-point between LTS and STS, Tb had a prevalence of 70.6% and 91.2% respectively (p=0.0018). Multivariable nominal logistic regression determined that tumour budding alone was an independent prognostic predictor of LTS in PDAC (*Table 1*).

Covariate	p-value
Presence of tumour budding	0.3652
Regional lymph nodes (pN)	0.2075
Primary Tumour Extent (pT)	0.2517
Presence of Lymphovascular Invasion	0.3663
Presence of Perineural Invasion	0.0291

* Table 1. Nominal logistic regression effect likelihood ratio test results

Conclusions:
While tumour budding appears to be able to differentiate these two groups of patients, its effect is lost in a multivariable approach. This is due to the fact that it shows a high degree of association with lymphovascular invasion.

REN JIE (ROBERT) YAO

Supervisor: Dr. Torsten Nielsen
Session: Basic Sciences

"Quisinostat and Bortezomib as a Potential Novel Therapeutic Combination in the Treatment of Synovial Sarcomas"

AUTHORS:
Ren Jie Robert Yao[3], Aimée N. Laporte[1,2,3], Le Su[1], Jennifer Ji[3], Angela Goytain[3], Neal Poulin[3], Torsten O. Nielsen[1,2,3]

AFFILIATIONS:
[1]University of British Columbia, Department of Pathology and Laboratory Medicine; [2]BC Cancer Agency; [3]Vancouver Coastal Health Research Institute

Background/objectives:
Found most commonly in adolescents and young adults, synovial sarcoma is an aggressive soft-tissue malignancy that makes up approximately 10% of soft-tissue sarcomas. While radiation and excision of the tumour are effective for controlling the local disease, metastatic disease is usually fatal due to the lack of effective chemotherapeutic options. Conventional chemotherapeutics such as doxorubicin and ifosfamide have limited benefits, and there are currently no molecularly targeted chemotherapeutics available for this cancer.

Synovial sarcomas contain the translocation-derived fusion oncoprotein SS18-SSX, which acts through transcriptional dysregulation and results in aberrant gene expression, such as the repression of tumour suppressors including EGR1. This study was conducted after an initial drug screen, which identified HDAC inhibitors, PI3K inhibitors and proteasome inhibitors as potential therapeutic options for synovial sarcomas.

Methods:
The initial drug screen was conducted with a 900 compound library against a panel of six synovial sarcoma cell lines, one breast cancer cell line and one normal embryonic kidney cell line. The top hits of the drug screen were verified and all the IC50 values were generated 48 hours post treatment. MTS assays were used to calculate cell viability, and Incucyte live cell imaging was used to qualitatively assess cell viability. Western blots were used to study the effects of the drugs on gene expression at the protein level to establish potential mechanisms of action and synergy. The synergy values between the drugs were computed using the Chou-Talalay method using CompuSyn software.

Results:
The top three classes of drugs identified by the screen were HDAC inhibitors, PI3K inhibitors and proteasome inhibitors. Within the different classes, quisinostat, BGT-226 and bortezomib were the most potent drugs. While quisinostat and BGT-226 did not synergize, quisinostat and bortezomib were found to synergize in all six synovial sarcoma cell lines. In addition, this synergy appeared to be selective for synovial sarcomas, since quisinostat and bortezomib did not synergize in the breast cancer and the normal embryonic kidney cell lines. Molecular validations showed a degradation of the SS18-SSX oncoprotein and a return in EGR1 expression, which adds support to a potential mechanism of synergy that is unique to synovial sarcomas.

Conclusions:
Quisinostat and bortezomib is a potential novel therapeutic combination for the treatment of synovial sarcoma. Future research is needed to further elucidate the mechanism of synergy, which may reveal novel pathways in the biology of synovial sarcomas.

JOHN LIM

Supervisor: Dr. Mari DeMarco
Session: Basic Sciences

"Designing a Quantitative Mass Spectrometric Assay for Transactive Response DNA Binding Protein 43"

AUTHORS:
John Lim[1], Emma Zheng[1] and Mari DeMarco[1,2]

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

Background/objectives:
We developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) workflow for the quantitation of TAR DNA-binding protein 43 (TDP-43). TDP-43 is a nuclear protein that controls gene expression in human cells. Recently, proteinacious inclusions of TDP-43 have been linked to neurodegenerative diseases including frontotemporal dementia and amyotrophic lateral sclerosis. Our long-term goal is to develop robust analytical tools to help evaluate TDP-43 as a potential biomarker.

Methods:
Via in silico tryptic digestion, structural characterization and proteomic database mining we identified candidate peptides for our selected reaction monitoring LC-MS/MS method. We applied a panel of selection criteria to select suitable peptides for analysis. Sample preparation for TDP-43 involved protein denaturation, alkylation and reduction, tryptic digestion and de-salting with C18 resin prior to LC-MS/MS. With a preliminary digest and LC-MS/MS protocol we were able to identify our four candidate tryptic peptides from TDP-43 and characterize at least three product ions per peptide. We then studied the effects of reduction and alkylation agents, trypsin type and digest time on the digest results (peak areas of precursor and product ions).

Results:
We identified 4 candidate tryptic peptides as a result of our selection process. We found that a combination of both dithiothreitol and iodoacetamide produced the most abundant signal; however, dithiothreitol alone was a close second and provided a shorter workflow. We found that TPCK trypsin produced the largest peak areas when digestions were longer than 20 hours. In testing various digest times (4, 6, 8, 10, and 24 hours) we found that production of tryptic peptides plateaued at 6 h.

Conclusions:
In conclusion, we have completed selection of candidate TDP-43 tryptic peptides to be used in a quantitative LC-MS/MS assay, optimized tuning of the MS, and characterized optimal digest conditions.

ADAM ZIADA

Supervisor: Dr. Hélène Côté
Session: Basic Sciences

"Blood Mitochondrial DNA Somatic Substitution Rates Increase with Age: Preliminary Analyses of a Cohort Study"

AUTHORS:
Adam S. Ziada[1,2], Jarek Ignas-Menzies, Meng Ying Lu[1,2], Beheroze Sattha[1,2], Hélène C.F. Côté[1,2] and the CIHR team grant on cellular aging and HIV comorbidities in women and children (CARMA).

AFFILIATIONS:
[1]Department of Pathology & Laboratory Medicine, UBC;
[2]Centre for Blood Research

Background/objectives:
Despite the success of antiretroviral therapy at preventing AIDS, there is mounting evidence that people living with HIV experience accelerated aging. Mitochondria, the powerhouse of the cell, contain their own mitochondrial DNA (mtDNA) which encodes proteins that are crucial to energy production and metabolism. The oxidative stress theory of aging states that the accumulation of mtDNA mutations over time leads to tissue aging and dysfunction. Somatic mtDNA point mutations are implicated in many age-associated diseases such as those seen in HIV+ individuals. The virus itself and the antiretroviral drugs can both negatively impact mtDNA as well as increase oxidative stress and may therefore contribute to accelerated aging.

Objective: In this cohort study, we investigated whether blood somatic mtDNA point mutations show a relationship with biological aging, HIV infection status, and smoking status.

Methods:
Somatic mtDNA point mutations are particularly challenging to quantify due to their low frequency. We developed a next generation mtDNA sequencing assay that can distinguish "true" mtDNA substitution mutations from background ones introduced through PCR or sequencing errors. The strategy is based on primers containing degenerate bases , generating 67 million unique mtDNA "tags" that are integrated into each molecule of mtDNA before they are amplified for sequencing on the Roche 454 platform. After sorting and aligning, mtDNA somatic mutation rates per 10,000bp are calculated. Adult HIV+ and HIV- study subjects were female participants enrolled in the CARMA cohort, who were HCV and HBV uninfected, as well as current or never smokers (but not past smokers). The pediatric subjects were randomly selected according to age group among 1) female HIV+ children enrolled in the CARMA cohort who had no perinatal exposure to HIV drugs and no detectable HIV plasma viral load, and 2) anonymous control children seen at BC Children Hospital and with leftover blood from routine testing. All children were assumed HCV and HBV-, as well as non-smoking. Comparisons of mtDNA mutation rates between groups were done using Mann-Whitney, while the association with age was analyzed by Spearman's correlation.

Results:
Blood mtDNA mutation rates were determined for 86 HIV+ and 62 HIV- individuals aged 1-75 years. A weak but significant association between mtDNA somatic substitution mutations and older age ($R = 0.243$, $p = 0.003$) was seen There was no significant association between mtDNA mutations and HIV status ($p = 0.173$) or smoking status ($p = 0.096$) although the observed effects were in the expected direction.

Conclusions:
Low mtDNA somatic mutation rates can be quantified in blood cells, despite the fact that this is a tissue with fast turnover. These preliminary analyses suggest that the blood mtDNA mutation rate increases with age, consistent with the current thinking on biological aging.

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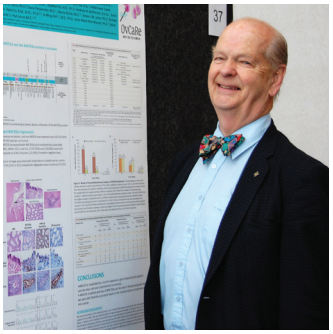

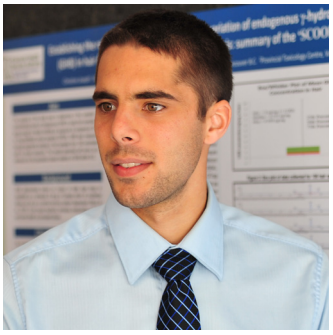

Dept of Pathology & Laboratory Medicine
UBC Hospital
Rm. G227 – 2211 Wesbrook Mall,
Vancouver, British Columbia
V6T 2B5 Canada
Phone: +1 604 822 7102
Email: pathologyday@pathology.ubc.ca
Website: www.pathology.ubc.ca

THE UNIVERSITY OF BRITISH COLUMBIA