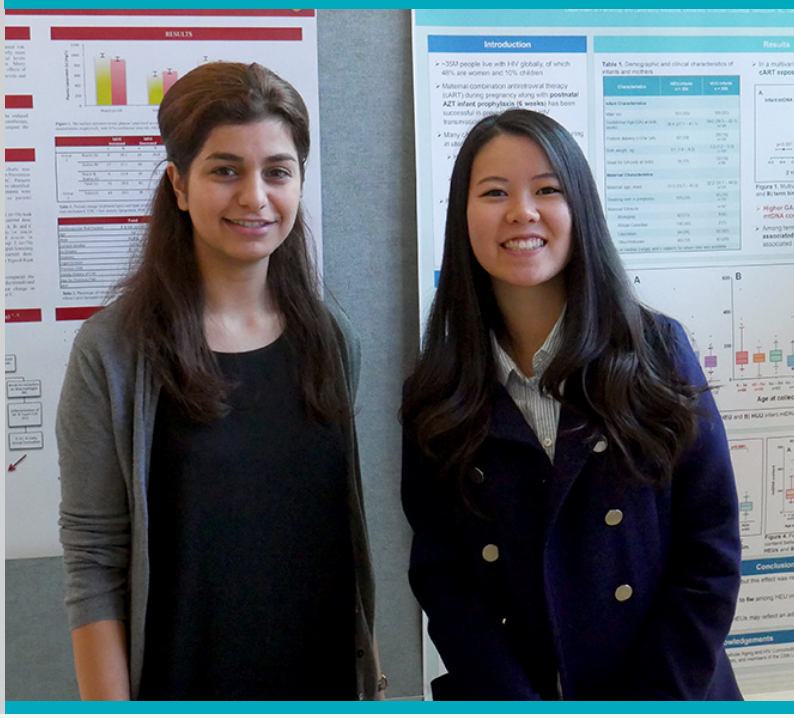
Abstract Book Posters & Oral Presentations

PATHOLOGY
laboratory medicine
FACULTY OF MEDICINE
UNIVERSITY OF BRITISH COLUMBIA

PATHOLOGY DAY

MAY 19, 2017

Featuring oral and poster presentations from graduate students, residents and others



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Let us tell you more about the amazing things we do:
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Acknowledgements

THE PATHOLOGY DAY COMMITTEE


Mike Allard


Corree Laule


Tony Ng


Avi Ostry


Yazeed Alwelaie


Brennan Wadsworth

WE HOPE YOU ENJOY PATHOLOGY DAY 2017.

THE PATHOLOGY DAY COMMITTEE:

MIKE ALLARD, CORREE LAULE, TONY NG, AVI OSTRY, YAZEED ALWELAIE, BRENNAN WADSWORTH

Pathology Day is a team effort and we would like to extend our thanks to everyone who contributed to the 2017 edition.

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

We also wish to express our gratitude to 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:

Reza Alaghehbandan
Mei Lin Bissonnette
Amanda Bradley
Sylvie Champagne
Nevio Cimolai
Helene Cote
Andy De Souza
Mari DeMarco
Katerina Dorovini-Zis
Maria Issa
Agatha Jassem
Steve Kalloger
Karuna Karunakaran
Niamh Kelly
Dailin Li
Hamid Masoudi
Bruce McManus
Jason Morin
Muhammad Morshed
Torsten Nielsen
John Priatel
Ed Pryzdial
Jacquie Quandt
Marc Romney
Ramesh Saeedi
Peter Schubert
Katherine Serrano
Junjan Shi
Jefferson Terry
Tracy Tucker
Peter van den Elzen
Bruce Verchere
Val White
Wei Xiong
Feng Xu

Finally, sincere thanks to the staff who kindly assisted with technical and administrative support throughout the day and our photographers: Heather Cheadle, Debbie Bertanjoli, Helen Dyck, Samantha Miller, Sandra Izzard, Dan Kim, Julie Ho and Jennifer Xenakis.

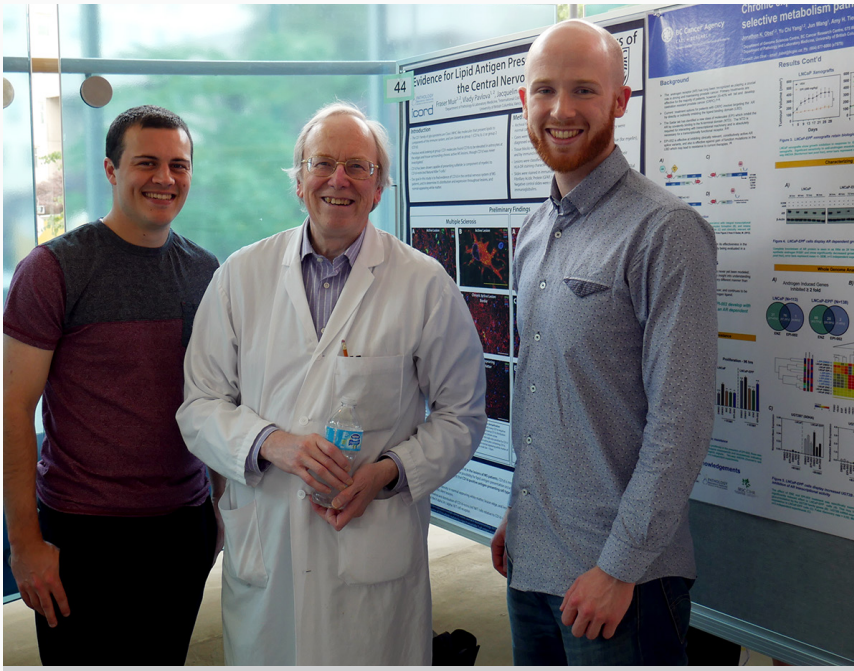
PATHOLOGY DAY
FRIDAY, MAY 19, 2017

MESSAGE

Pathology Day is a 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 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. Bruce McManus will give the James Hogg Lecture, while Dr. Jeffrey Ross (the Cyrus Strong Merrill Professor and Chair of the Department of Pathology and Laboratory Medicine at Albany Medical College) is our Keynote Speaker. 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, Dr. Tony Ng, Dr. Yazeed Alwelaie, Brennan Wadsworth, Cristina Low, and Adeline Chan, as well as all the other individuals whose efforts make the day a success.

DR. DON BROOKS
ACTING HEAD



KEYNOTE SPEAKER:
DR. JEFFREY ROSS

"COMPREHENSIVE GENOMIC PROFILING AND IMMUNOTHERAPY"

SPONSORED BY: Roche

The talk will focus on immuno-oncology with emphasis on approved indications (NSCLC, bladder cancer, melanoma, Merkel Cell, HNSCC, and lymphomas), biomarkers of efficacy (BRAF, MET exon 14 mutations) and resistance (STK11) including hyperprogression (MDM2/4) associated with checkpoint inhibitors.

CONFERENCE OUTLINE

Oral presentations and guest speakers will be at the Cordula and Gunter Paetzold Health Education Centre, 1st floor, Jim Pattison Pavillion North, Vancouver General Hospital

+ PAETZOLD AUDITORIUM & ATRIUM AT VGH

7:45 am	Breakfast
8:00 am	Opening remarks – Dr. Donald Brooks, Acting Department Head

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)

8:10 am – 8:25 am	Samantha Burugu
8:25 am – 8:40 am	Fraser Muir
8:40 am – 8:55 am	Stephanie Santacruz

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)

8:10 am – 8:25 am	Nissreen Mohammad (AP)
8:25 am – 8:40 am	Daniel Owen (AP)
8:40 am – 8:55 am	Jessica Saunders (AP)

9:00 AM – 9:20 AM GUEST SPEAKER

DR. VALERIO RUSSO
Granzyme B: an important contributor to dermal-epidermal separation in blistering skin conditions

Dr. Valerio Russo is a Post-doctoral fellow at the iCord Research Centre, Dr. Granville's Lab.

**Post-doctoral fellow – the third faculty oral presentation spot is awarded to the highest rated abstract in the post-doctoral fellow category.*

09:25 AM – 09:45 AM GUEST SPEAKER

DR. JULIE IRVING
Academic and Community Practice in Anatomical Pathology: An Oxymoron?

Dr. Julie Irving is a Clinical Associate Professor in the Department of Pathology & Laboratory Medicine at the University of British Columbia. She was an Anatomical Pathologist in the Department of Pathology and Laboratory Medicine at the Vancouver General Hospital from 2004-2007. Since 2007, Dr. Irving has practiced subspecialty and consultation Gynecological Pathology at the Royal Jubilee Hospital in Victoria, BC.

09:45 AM – 10:00 AM BREAK (ATRIUM)

NEW 10:00 AM – 10:30 AM POSTER POWER PITCH SESSION

10:35 AM – 11:35 AM JAMES HOGG LECTURE

DR. BRUCE MCMANUS
Human Suffering - Crossroads of the 7 C's

Dr. Bruce McManus is a Professor in the Department of Laboratory Medicine at the University of British Columbia. He serves as CEO, Centre of Excellence for Prevention of Organ Failure (PROOF Centre) and Co-Director, Institute for Heart + Lung Health.

CONFERENCE OUTLINE

Poster session and awards reception will be at ICORD, Blusson Spinal Cord Centre, 818 West 10th Ave

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)

11:40 am – 11:55 am	Nicha Boonpattrawong
11:55 am – 12:10 pm	Jon Obst
12:10 pm – 12:25 pm	Wenchen Zhao

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)

11:40 am – 11:55 am	Sarah Cherian (MM)
11:55 am – 12:10 pm	Lisa Li (MM)
12:10 pm – 12:25 pm	Maryam Al Bakri (HP)

12:30 PM – 2:30 PM POSTER SESSION & LUNCH AT ICORD

2:45 PM – 3:05 PM GUEST SPEAKER

DR. PHILIPP LANGE
Proteome Complexity - Challenge and Opportunity for Precision Medicine

BC Children's Hospital Research Institute

Dr. Philipp Lange is an Assistant Professor in the Department of Pathology and Laboratory Medicine at the University of British Columbia and Investigator at the BCCHRI and BCCRC. As Canada Research Chair in 'Translational Proteomics of Pediatric Malignancies' he heads an integrated proteomics and bioinformatics driven research program investigating the molecular basis and new treatment avenues in childhood cancer.

GRADUATE STUDENT ORAL SESSION (LECTURE THEATRE)

3:10 pm – 3:25 pm	Maryam Ghaedi
3:25 pm – 3:40 pm	Bryan Lin
3:40 pm – 3:55 pm	Alberto Delaidelli

RESIDENT ORAL SESSION (MULTIPURPOSE ROOM)

3:10 pm – 3:25 pm	Tyler Hickey (AP)
3:25 pm – 3:40 pm	Yazeed Alwelaie (AP)
3:40 pm – 3:55 pm	Kyra Berg (AP)

3:55 PM – 4:10 PM BREAK (ATRIUM)

4:10 PM – 5:10 PM KEYNOTE SPEAKER

DR. JEFFREY ROSS
Comprehensive Genomic Profiling and Immunotherapy

FOUNDATION MEDICINE

Dr. Jeffrey Ross is the Cyrus Strong Merrill Professor and Chair of the Department of Pathology and Laboratory Medicine at Albany Medical College. He is the medical director of Foundation Medicine and a leader in the field of molecular diagnostics.

5:30 PM COCKTAILS & CANAPES (ICORD)

6:00 PM AWARDS (ICORD) EVENING EVENT CLOSES AT 8:30 PM

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14	JON OBST	JON OBST[1], JUN WANG[1], DAVID WILLIAMS[2], AMY TIEN[1], NASRIN MAWJI[1], YU CHI YANG[1], RAYMOND ANDERSEN[2], MARIANNE SADAR[1] Chronic exposure to a novel androgen receptor N-terminal domain inhibitor induces resistance via a selective metabolism pathway
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20	KING MONG (REBECCA) TONG	KING MONG TONG[1], GHADA AL-RAWAHI[2], JOSEPH TING[3], DAVID GOLDFARB[3], VANESSA PAQUETTE[4], KRISTOPHER KANG[3], HORACIO OSIOVICH[3], ASHLEY ROBERTS[3] Urinary tract infection in premature neonates: A 5-year retrospective study
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23	ELLIA ZHONG	ELLIA ZHONG[1], JOHN DE GUZMAN[1], JULIE HO[1], AMY LUM[2], STEPHEN YIP[1], TONY NG[1] Exploration of NRG1 fusions and other genomic drivers in cholangiocarcinoma
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#	NAME	POSTER PRESENTATIONS
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69	YU-HSUAN HUANG	YU-HSUAN HUANG[1,2], KEVIN TSAI[1,2], SOHYEONG KANG[1,2], MANDY FORD[3], MARC HORWITZ[4] AND JOHN PRIATEL[1,2] The role of SAP and 2B4 CD48/interaction in B cell immunosurveillance by effector CD8 T cells

#	NAME	POSTER PRESENTATIONS
70	TAYYEBEH NAZERAN	MICHAELANGLESIO[1,2], NICHOLAS PAPADOPOULOS[3], TAYYEBEH NAZERAN[2,6], AYSE AYHAN[4,5,7,8], HUGO HORLINGS[6,9], MICHAEL NOEL[5], AMY LUM[9], SIÂN JONES[10], JANINE SENZ[2], TAMER SECKIN[11], JULIE HO[6], REN-CHIN WU[12], VIVIAN LAC[6,8], HIROSHI OGAWA[7], BASILE TESSIER-CLOUTIER[2,6], RAMI ALHASSAN[11], AMY WANG[6], YUXUAN WANG[4], FONTAYNE WONG[13], ADNAN HASANOVIC[11], NATASHA ORR[13], MING WANG[4], MARIA POPOLI[3], JOY SCHAEFFER[3], WYATT MCMAHON[3], LAURA WOOD[5], AUSTIN MATTOX[3], CATHERINE ALLAIRE[1,13], JAMES SEGARS[4], CHRISTINA WILLIAMS[1,13], CRISTIAN TOMASETTI[3], NIKI BOYD[9], KENNETH KINZLER[3,] C. BLAKE GILKS[2,6], LUIS DIAZ[3,8], TIAN-LI WANG[3,4,5], BERT VOGELSTEIN[3], PAUL YONG[1,13], DAVID HUNTSMAN[1,2,6,9,] AND IE-MING SHIH[3,4,5], Cancer associated mutations in non-cancer associated endometriosis
71	TAYYEBEH NAZERAN	TAYYEBEH NAZERAN[1,2], ANGELA CHENG[1,3], ANTHONY KARNEZIS[1,2], ANNA TINKER[4], BLAKE GILKS[1,4] Bartholin gland carcinoma :p16 expression and clinical outcome
72	VALERIO RUSSO (**ORAL PRESENTATION**)	VALERIO RUSSO[1,2], THEO KLEIN[3,4], NICK CARR[5], NANCY VAN LAEKEN[5], RICHARD CRAWFORD[6], CHRIS OVERALL[3,4], DAVID GRANVILLE[1,2] Granzyme B: an important contributor to dermal-epidermal separation in blistering skin conditions
73	JUNYAN SHI	JUNYAN SHI[1], EMMA ZHENG[1], MARI DEMARCO[1,2] Quantification of c-reactive protein in plasma by high performance liquid chromatography tandem mass spectrometry
74	CHRISTOPHER TURNER	CHRISTOPHER TURNER[1], HONGYAN ZHAO[1], PHILIP BIRD[2], ANTHONY PAPP[3], DAVID GRANVILLE[1] Granzyme K impairs burn wound healing
75	FENG XU	FENG XU[1], DRAGOS VASILESCU[1], DAISUKE KINOSE[1], NAOYA TANABE[1], BIN LIU[2], JAKE KANTROWITZ[3], STIJN VERLEDEN[4], BART VANAUDENAERDE[4], JOEL COOPER[5], MARC LENBURG[3], AVRUM SPIRA[3], RAYMOND NG[2], JIM HOGG[1] The molecular difference between centrilobular and panlobular emphysema
76	YUZI ZHENG	YU ZI ZHENG[1], TERRY WONG[1], PAWAN DHALIWAL[1], MARI DEMARCO[1,2] Mass spectrometric identification of the plasma ACTH-ome
77	HAMISH NICOLSON	HAMISH NICOLSON[1], LAWRENCE SHAM[2], KRISTINE ROLAND[3] Prophylactic antigen matched donor blood for patients with warm reactive igg autoantibodies: an evaluation of safety and efficacy
78	WAREN BATICADOS	*WAREN BATICADOS[1,2], CHELSEA HIMSWORTH[3,4], AGATHA JASSEM[1,2], SHING ZHAN[6], SEPIDEH ALAMOUTI[6], JUAN DUAN[1,2], MICHELLE COOMBE[3,4], PATRICK TANG[5], BRIAN KWOK[6], MOHAMMAD QADIR[6], WILLIAM HSIAO[1,2] AND NATALIE PRYSTAJECKY[1,2]* Wild waterfowl-independent avian influenza virus surveillance and characterization using real-time PCR and enrichment-based targeted resequencing of wetland sediments
79	SOPHIE STUKAS	SOPHIE STUKAS[1], VICTORIA HIGGINS[2], KHOSROW ADELI[2], CHERYL WELLINGTON[1] Generation of paediatric reference intervals for blood detection of the brain protein tau

NISSREEN MOHAMMAD

- *RESIDENT*
- *ORAL SESSION*
- *SUPERVISOR: DR. TONY NG*

THE UTILITY OF MOLECULAR CHARACTERIZATION OF GYNECOLOGIC SMALL BLUE ROUND CELL TUMORS: REPORT OF TWO EWSR-1ERG-POSITIVE EWING SARCOMA CASES

Nissreen Mohammad[1], Angela Goytain[2], Martin Jones[3], Shangguo Tang[4], Michael Brennan[5], Blake Gilks[1,6], Janessa Laskin[7], Tony Ng[1,6]

BACKGROUND/OBJECTIVES: Ewing sarcoma (ES) is molecularly characterized by translocations most commonly resulting in fusion of the EWSR1 gene with FLI1 or ERG. Distinguishing ES from other sarcoma types is critical because it portends a specific course of aggressive systemic therapy. ES of the gynecologic tract is rare, with overlapping morphologic, immunohistochemical and molecular features with other pelvic small round blue cell tumors (SBRCTs). Here, we discuss two cases of gynecologic SBRCTs with EWSR1-ERG fusion.

METHODS: Two cases of pelvic small round cell tumors were subjected to molecular characterization by a Nanostring-based sarcoma fusion gene assay developed in house. One case was also subjected to whole genome/transcriptome analysis through the local Personalized Oncogenomics project. Results were validated using FISH and RTPCR.

RESULTS: Case 1 was a 21-year-old female with a large ovarian tumor, and Case 2 a 27-year-old female with a large pelvic mass and extensive intra-abdominal and lung metastases. Both tumors were positive for CD99 and CAM5.2. Nanostring assay performed on Case 1 showed expression of the EWSR1-ERG fusion. The result was validated by FISH and RTPCR. Case 2 underwent whole genome/transcriptome analysis; the EWSR1-ERG fusion was identified along with various other molecular alterations, including a pathogenic STAG2 mutation commonly seen in aggressive cases of ES. EWSR1-ERG expression was validated using the Nanostring assay. Case 2 initially received radiation and gynecologic chemotherapy protocol with poor response. Once the final diagnosis was reached, she was switched to ES protocol and showed near complete response.

CONCLUSIONS: We report two cases of keratin-positive, EWSR1-ERG-positive Ewing sarcoma of the gynecologic tract; characterization of the fusion oncogene lead to correct diagnosis, which was not suspected prior to molecular analysis. Pelvic ES is rare, and identification of both fusion gene partners was needed to rule out other differential diagnoses, particularly desmoplastic small round cell tumor, as well as other gynecologic SRBCTs.

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DANIEL OWEN

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LOSS OF BRCA-1ASSOCIATED PROTEIN 1 (BAP1) EXPRESSION IS RARE IN NON-SMALL CELL LUNG CANCER

Daniel Owen[1], Brandon Sheffield[2], Diana Ionescu[3], Andrew Churg[1]

BACKGROUND/OBJECTIVES: BRCA1-associated protein 1 (BAP1) is a tumor suppressor gene involved in regulation of the cell cycle, cellular differentiation, repair of DNA damage, and apoptosis. In the distinction of malignant mesothelioma from benign mesothelial proliferations, immunohistochemical loss of BAP1, the protein expressed by the BAP1 gene, has proven highly specific for malignant mesothelioma. However, few studies have investigated the rate of BAP1 loss in tumors that commonly metastasize to the pleura. Our objective is to determine the rate of BAP1 loss in non–small cell lung cancer (NSCLC).

METHODS: Immunohistochemistry for BAP1 was performed using tissue microarrays containing 133 confirmed cases of NSCLC (80 of lung adenocarcinoma and 53 of squamous cell carcinoma). Cases were interpreted as showing BAP1 loss if nuclear staining was completely absent in all tumor cells and present in stromal and inflammatory cells that served as internal controls. Cases showing no BAP1 staining in the internal controls were excluded.

RESULTS: After exclusion of 32 cases for technical reasons, only 1 case of pulmonary adenocarcinoma of 101 cases of NSCLC (69 adenocarcinoma and 32 squamous cell carcinoma; 1.0% of cases) showed BAP1 loss.

CONCLUSIONS: We conclude that loss of BAP1 expression is a rare event in NSCLC. Therefore, BAP1 is a potentially useful addition to the immunohistochemical markers used to distinguish mesothelioma from pleural metastasis of NSCLC.

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JESSICA SAUNDERS

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A CASE REPORT OF A NOVEL MUTATION IN TTC7A INVOLVING A HOMOZYGOUS MUTATION IN EXON 15 AND A HETEROZYGOUS MUTATION IN EXON 15-12 CAUSING FAMILIAL MULTIPLE INTESTINAL ATRESIA WITH SEVERE COMBINED IMMUNODEFICIENCY

Jessica Saunders[1], Jonathon Bush

BACKGROUND/OBJECTIVES:
Case report

METHODS: N/A

RESULTS: N/A

CONCLUSIONS: In the past several years, multiple centers have reported autosomal recessive mutations in tetratricopeptide repeat domain 7A (TTC7A) gene in patients with mulitple intestinal atresia and immunodeficiency. Here, we present an infant with multiple intestinal atresia and combined immunodeficiency who was revealed to carry two deletions within the TTC7A gene by targeted deletion and duplication analysis. The first corresponds to a heterozygous deletion of intron 11 to intron 15 (exons 12-15). The second deletion corresponds to a homozygous deletion of intron 14 to 15 (exon 15).

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SARAH CHERIAN

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EVALUATION OF THE BRUKER BIOTYPER MATRIX ASSISTED LASER DESORPTION IONIZATION- TIME OF FLIGHT SUBTYPING MODULE FOR THE DETECTION OF METHICILLIN- RESISTANT STAPHYLOCOCCUS AUREUS

Sarah Cherian[1], Kennard Tan[1,2]

BACKGROUND/OBJECTIVES: Methicillin resistant Staphylococcus aureus (MRSA) is an organism commonly encountered in the clinical microbiology laboratory, with significant healthcare implications. Matrix assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF MS) is increasingly being used in clinical laboratories for the rapid identification of organisms. The Bruker Biotyper MALDI-TOF MS have recently released a subtyping module that detects the presence of MRSA.

METHODS: Clinical samples were subcultured to appropriate agar media, according to the laboratory's routine procedures. After overnight incubation, colonies resembling Staphylococcus aureus were transferred to a target plate and identification was confirmed using a Bruker Biotyper (Bruker Daltonics, Billerica, MA, USA) MALDI-TOF MS system in accordance with the manufacturer's recommendations. All organisms that were identified as Staphylococcus aureus underwent antimicrobial susceptibility testing using the BD Phoenix Automated Microbiology System (BD, Sparks, MA, USA), according to the manufacturer's recommendations. The BD Phoenix system has been previously evaluated for the identification of MRSA.

RESULTS: 925 unique isolates of S. aureus were analysed. The MALDI- TOF MS subtyping module showed a sensitivity of 0.5(0.44-0.56), specificity of 0.89 (0.86- 0.91), positive predictive value of 0.64 (0.57-0.70), negative predictive value of 0.82(0.79- 0.85).

CONCLUSIONS: While the sensitivity of the Bruker biotyper subtyping module for the detection of MRSA is low, the high specificity makes it an excellent tool for rapid detection of MRSA which will greatly improve treatment and infection control outcomes.

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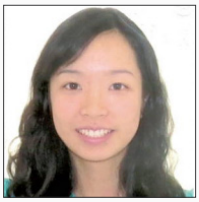

LISA LI

- *RESIDENT*
- *ORAL SESSION*
- *SUPERVISOR: DR. MICHAEL PAYNE, DR. CHRIS LOWE*

HIV SEROLOGY SIGNAL-TO-CUTOFF RATIO AS A RAPID METHOD TO PREDICT CONFIRMATION OF HIV INFECTION

Li Lisa[1], Puddicombe David[2], Champagne Sylvie[1,3], Jassem Agatha[1,4], Krajden Mel[1,4], Lowe Chris[1,3], Payne Michael[1,3]

BACKGROUND/OBJECTIVES: To assess the ability of signal-to-cutoff (S/CO) ratio from 4th generation HIV serology to predict subsequent confirmation of HIV infection.

METHODS: Patients from August 2012 to August 2016 with a new positive HIV serology (S/CO>1) were included. Serology was performed using the Abbott Architect HIV Ag/Ab Combo assay. S/CO ratios were compared to the results of subsequent confirmatory testing at a reference laboratory, which consisted of: repeat serology testing, HIV-1 RNA NAAT and HIV-1 Western blot. Predictive probabilities (PPs) of a positive confirmatory result were calculated based on a logistic regression model.

RESULTS: 250 patients were included, comprising 84 (34%) HIV negative patients, 136 (54%) chronic infections, and 30 (12%) acute infections. Higher S/CO values were associated with increased odds of confirmed infection (odds ratio 1.18, 95% CI 1.09-1.28, p<0.001). The PP of a confirmed positive result increased with higher S/CO values.

CONCLUSIONS: Signal-to-cutoff values were strongly associated with confirmed HIV infection, with a PP of 100% at a S/CO of 50. These results enable a more informed discussion of S/CO thresholds for reporting preliminary HIV infection prior to a confirmatory result. Patient risk factors and clinical symptoms should also be taken into account but were not available in this study. Earlier diagnosis of HIV, particularly for acute seroconversions, allows earlier initiation of treatment and public health interventions, which can help reduce transmission.

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MARYAM AL BAKRI

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MONITORING UNFRACTIONATED HEPARIN THERAPY: A COMPARISON BETWEEN TWO CHROMOGENIC ANTI-FACTOR-XA ASSAYS

Maryam Al Bakri[1], Nicholas Au[1,2], Mykola Maydan[2]

BACKGROUND/OBJECTIVES: Unfractionated heparin is used in critically ill pediatric patients on extracorporeal life support (ECLS) or for initial treatment of thromboembolic events when low molecular weight heparin is not suitable. Anti-Xa measurements is often considered a 'golden standard' for laboratory-based monitoring of heparin treatment, however the composition of anti-Xa reagents varies. In this study, we aim to systematically determine the agreement between two different chromogenic anti-Xa assays, HemosIL liquid anti-Xa (reagent with dextran sulfate) and STA-Liquid Anti-Xa (reagent without dextran sulfate) in the pediatric population.

METHODS: Samples from pediatric patients receiving therapeutic unfractionated heparin for ECLS and thromboembolic diseases were obtained prospectively from February 2016 to December 2016. Samples from adults on unfractionated heparin were also obtained from St. Paul's Hospital for comparison. All samples were analyzed using HemosIL from Instrumentation Laboratory (IL) and STA-Liquid Anti-Xa from STAGO Company. The testing was performed according to manufacturer's instructions using the BCS® XP System (Siemens) for both assays at BCCH. In order to assess any possible instrument effect, we repeated the testing on ACL TOP 700 IL using HemosIL reagent at VGH. The correlation between the two assays was assessed using regression analysis and the agreement was tested using Bland-Altman plot analysis.

RESULTS: 47 samples from 11 patients were obtained. The correlation between HemosIL and STA anti-Xa using the BCS XP was poor (r=0.86, y = 1.2809x + 0.1326). The agreement between these two assays was poor as well with a mean bias of 0.21;(95% limits of agreement 0.571 & -0.144). There was a constant positive Bias where HemosIL gave higher results (96% of all samples). 15/47 (32%) gave discordant results within the therapeutic range (0.3-0.7 IU/ml). In contrast, the correlation between the two assays when adult samples were tested was higher (r=0.96), but a mean positive bias of 0.14 is also present. To evaluate any inter-instrument variability, we compared the results from two different analyzers using the same reagent HemosIL. The results from ACL TOP 700 and BCS XP correlated very well (r >0.980, y = 1.0774x + 0.0017). The agreement between the results was excellent; mean Bias 0.04, 95% limits of agreement (-0.098-0.174). There was 93% concordance of results within therapeutic range.

CONCLUSIONS: Our study supports the previous findings of significant variability between different anti-Xa assays. However, it showed that this variability is more evident in pediatric population compared to adults. This is not explained entirely by reagent or instrumentation effect. A contributor to the poor agreement includes different reagent constituents, e.g. dextran sulfate, but this would not account for the poorer comparability seen in pediatric samples. The dynamic hemostatic system in children may contribute to this difference. It will be interesting to investigate if there will be any clinical consequences implicated by the variability between these two assays.

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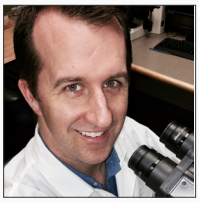

TYLER HICKEY

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IATROGENIC HYDROPHILIC POLYMER EMBOLI FOLLOWING ENDOVASCULAR PROCEDURES: A POTENTIALLY FATAL COMPLICATION

Tyler BM Hickey[1,2], Avrum J Ostry[1,4], Jason B Chew[5,6], James Caldwell6, Michael A Seidman[4], Hamid Masoudi[1,4] and John A Maguire[1,3]

BACKGROUND/OBJECTIVES: Hydrophilic polymer embolization (HPE) has recently been described as a complication of the polymer coating used on many investigative and interventional endovascular devices. Several recent case reports and small case series have described embolization of this distinct foreign material to a variety of tissues with consequences that ranged from being asymptomatic to fatal; e.g. from stroke. HPE material is described as lightly basophilic, amorphous and/or lamellated, non-polarizable, non-refractile, intravascular foreign substance. Awareness of this potential complication led to this retrospective clinicopathologic autopsy analysis with pre-mortem diagnostic imaging correlation.

METHODS: Review of the Vancouver Coastal and Providence Health Laboratory databases from 2010-2016 identified individuals who had undergone endovascular procedures using polymer-coated endovascular devices within 3 months of death. Diagnostic imaging studies were reviewed by two Radiologists. Autopsy slides were blindly reviewed by three Pathologists, a Neuropathologist, and a Senior Pathology Resident.

RESULTS: The average patient age in the 100 autopsies was 64.5 years (range 19-95) with 65% males and 35% females. We assessed an average of 35 glass slides per autopsy. Of the autopsies included in our study, 96% were unrestricted hospital autopsies including examination of the head, thoracic, abdominal and pelvic organs, while 4% were heart only. We identified histologic evidence of HPE in 23% of patients who had a hospital autopsy after receiving any type of endovascular procedure within 90 days of death. There was radiologic evidence of infarction in 26% of cases with HPE histology. The involved organs included: kidneys (n=12), heart (n=8), lungs (n=8), spleen (n=4), brain (n=2), liver (n=2) and single findings in the pancreas, colon, stomach and adrenal gland. Endovascular aortic repairs showed the greatest density/distribution of HPE. Also, HPE material shows degradation with time and is sometimes associated with an inflammatory response including foreign body giant cells and lymphocytes.

CONCLUSIONS: We present the largest documented collection of HPE findings to date. We have observed that deposition of HPE can result from various types of endovascular procedures. These iatrogenic emboli may result in tissue injury via occlusive ischemia/infarction. Also, HPE may occur in a wide range of organs and the appearance of this foreign material seems to change over time, possibly due to host immune response. In spite of endovascular procedures often being performed for life-saving efforts, it is recognized that deposition of HPE to sensitive sites (e.g brain, heart) may independently contribute to patient morbidity and mortality. We advocate for greater awareness of this important and occasionally fatal complication of endovascular procedures.

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YAZEED ALWELAIE

- *RESIDENT*
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PRIMARY ALK-POSITIVE LUNG ADENOCARCINOMA IN A TEENAGER TREATED FOR RELAPSED NEUROBLASTOMA

Alwelaie YA[1], Deyell RJ[2], Nadel HR[3], Tucker T[1], Laskin J[4], Rassekh R[2], Zhou C[5], English JC[1], Lee AF[1]

BACKGROUND/OBJECTIVES: Primary adenocarcinoma of the lung is exceedingly rare in the pediatric population, but has been reported in patients with non-pulmonary childhood cancers. Herein, we report a case of a 17-year-old boy of East Asian ancestry, initially diagnosed with stage IV unfavorable histology neuroblastoma at the age of 6. He underwent standard therapy for high-risk neuroblastoma but had multiple relapses, each treated with standard therapy for relapsed neuroblastoma. From age 11-15 he took etoposide, an oral metronomic agent, which was discontinued at age 15 following negative meta-iodobenzylguanidine (MIBG) scans for >1 year. At age 16, routine imaging follow-up demonstrated a new, MIBG-avid sphenoid mass consistent with relapsed neuroblastoma, for which he received focal radiation therapy. Staging evaluation showed a single cluster of neuroblastoma in the marrow along with one focal area of MIBG-avid relapse in the sphenoid (Curie score 1). Also identified on the CT chest were multiple, small (<5mm), non MIBG-avid lung nodules, 9 in the right lung and 4 in the left lung. On review of past imaging, several small pulmonary nodules were evident on prior MIBG/CT imaging up to over 3 years earlier. As neuroblastoma is unusual in the lung and because the patient had no evidence of infectious symptoms, an interventional radiology-guided thoracoscopic lung wedge resection was pursued. Pathologic analysis of one of the nodules revealed a 3 mm focus of predominantly lepidic growth with central desmoplastic reaction, best classified as “minimally invasive adenocarcinoma” (MIA). Multiple microscopic foci of atypical adenomatous hyperplasia (AAH) were also identified in the background lung parenchyma. There was no evidence of metastatic neuroblastoma in the sample. Immunohistochemical staining for ALK protein was positive in the MIA, but negative in the AAH. ALK gene rearrangement in the MIA was confirmed by fluorescence in situ hybridization (FISH). Surveillance recommended by multidisciplinary Tumour Board has shown stability of the pulmonary nodules over 6 months. No lung cancer directed therapy has been initiated, but the patient has undergone systemic therapy for neuroblastoma with progressive disease following cyclophosphamide/topotecan (Curie score 8), and subsequently a partial response to immunotherapy with chimeric antibody 14.18/irinotecan/temozolomide (Curie score 2). Evaluation by Hereditary Cancer program has not identified any underlying germline cancer predisposition. This report aims to highlight that although pediatric primary lung carcinomas are rare, they should be considered in the setting of new lung nodules that arise following intensive treatment for non-pulmonary pediatric cancers such as neuroblastoma.

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KYRA BERG

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GATA3 IMMUNOHISTOCHEMISTRY FOR DISTINGUISHING SARCOMATOID AND DESMOPLASTIC MESOTHELIOMA FROM SARCOMATOID CARCINOMA OF THE LUNG

Kyra Berg[1], Andrew Churg[1,2]

BACKGROUND/OBJECTIVES: The separation of sarcomatoid and desmoplastic malignant mesotheliomas from sarcomatoid carcinomas of the lung metastatic to the pleura can be difficult, since both types of tumor can be morphologically similar and are frequently positive only for pan-keratin. GATA binding protein 3 (GATA3) is most commonly used as an immunohistochemical marker of breast and urothelial carcinoma, but has also been reported to stain other types of tumors including some mesotheliomas. In this study we asked whether GATA3 immunohistochemistry could be used to distinguish sarcomatoid/desmoplastic malignant mesotheliomas from sarcomatoid carcinomas of the lung.

METHODS: Cases of sarcomatoid/desmoplastic malignant mesotheliomas (N=19) and sarcomatoid carcinomas of the lung (N=13) were selected retrospectively and stained with GATA3. Tumor staining was scored for diffuseness and intensity, with a maximum possible score of 6.

RESULTS: All 19 sarcomatoid/desmoplastic malignant mesotheliomas examined showed strong diffuse staining for GATA-3 (no case scored <3, mean score ± SD for all 19 cases 5.4± 0.9), whereas only 2/13 sarcomatoid carcinomas of the lung stained positively for GATA3 and the staining was weak and patchy (score 2 for each case, mean ± SD for all 13 cases 0.4±0.8).

CONCLUSIONS: Any positive staining for GATA-3 was 100% sensitive and 85% specific for sarcomatoid/desmoplastic mesothelioma. We conclude that strong diffuse staining for GATA-3 favors a diagnosis of sarcomatoid/desmoplastic malignant mesothelioma over metastatic sarcomatoid carcinoma of the lung.

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SAMANTHA BURUGU

- *GRADUATE STUDENT*
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THE IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT INTERPLAY IN BREAST CANCER PATIENTS

Samantha Burugu[1,2], Dongxia Gao[2], Christine Chow[2] and Torsten O. Nielsen[1,2]

BACKGROUND/OBJECTIVES: Cancers have evolved immune evasion mechanisms to promote their survival such as aberrant expression of immunosuppressive markers. Targeting immunosuppressive markers has led to clinical success with the example of immune checkpoint inhibitors. New immunosuppressive targets include the enzyme indoleamine 2,3-dioxygenase (IDO-1) and the cytokine Interleukin-23 (IL-23). IDO-1 catabolizes tryptophan leading to impaired T-cell proliferation and is mainly expressed by antigen presenting cells. IL-23 is produced by macrophages and its function in cancer is still unclear but may have tumor-promoting activity. In what to our knowledge is the first study of its kind, we investigated the expression and prognostic value of IDO-1 and IL-23 in breast cancer patients.

METHODS: A tissue microarray (TMA) of 330 breast cancer excision specimens linked to detailed clinical data was used to assess IDO-1 and IL-23 expression by immunohistochemistry. TMA sections were stained with IDO-1 clone SP260 or IL-23 clone HLT2736. Immune cells (mostly macrophages) expressing IDO-1 or IL-23 were scored following their localization (stromal or intra-epithelial) and reported as absolute counts per core. Positive carcinoma cells were reported by staining intensity per core. CD68 and CD163 biomarkers were stained on the TMA to assess the functional state of tumor-associated macrophages. All statistical and survival analyses were conducted using SPSS.

RESULTS: We found IDO-1+ stromal macrophages in 22% and intra-epithelial macrophages in 11% of breast cancers. IDO-1+ carcinoma cells were present in 6% of cases. 56% of breast cancers had high levels of IL-23+carcinoma staining and one case had IL-23 staining on macrophages. IDO-1+ macrophages or carcinoma cells were significantly associated with major risk factors (estrogen receptor (ER) negativity and high Ki67 proliferation index) and were enriched in the aggressive basal-like breast cancer subtype. High IL-23+ carcinoma staining was not associated with any of the clinical parameters tested. In survival analyses, IDO-1+ macrophages were a biomarker for significantly improved survival among ER- patients. High IL-23+carcinoma staining was significantly associated with improved survival (in the whole cohort and in ER+ patients). Moreover, 90% of cases with IDO-1+ macrophages were co-infiltrated with pro-inflammatory CD68+ macrophages. In contrast, only 33% of cases with IDO-1+ macrophages were co-infiltrated with anti-inflammatory CD163+ macrophages. Surprisingly, high IL-23+ carcinoma staining was significantly associated with CD68+ macrophages and not CD163+ macrophages.

CONCLUSIONS: Although both IDO-1 and IL-23 are associated with immunosuppressive activity, our findings show that in breast cancer, their expression is enriched in patients with better survival which may indicate that they serve to mark ongoing immune activity. Our findings may have clinical implications as IDO-1 inhibitors are being evaluated in clinical trials, and IL-23 inhibitors are showing promise potential in preclinical cancer studies. Validation of the findings on an independent cohort powered for multivariate analyses is underway.

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FRASER MUIR

- *GRADUATE STUDENT*
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LIPID ANTIGEN PRESENTATION IN THE CENTRAL NERVOUS SYSTEM IN MULTIPLE SCLEROSIS

Fraser Muir[1,2], Jacqueline Quandt[1], G. R. Wayne Moore[1, 2, 3]

BACKGROUND/OBJECTIVES: The inflammatory immune response has long been held to be a key component to the disease process of Multiple Sclerosis (MS), which is believed to be autoimmune in nature. Research conducted in our lab looking at the diffusely abnormal white matter in archival MS brain tissue has shown that there is a lipid-specific depletion with preservation of myelin proteins within the diffusely abnormal white matter, implicating a response against myelin lipids in MS. The lipid antigen presenting molecules of the immune system are the five members of the CD1, Class I MHC-like, protein family. Of these five CD1d has been shown to present sulfatide (a component of myelin) to immune cells such as natural killer T cells. Human studies have found changes in the regulation and number of circulating monocytes expressing CD1 in MS and implicate them in the disease process, yet to date no studies have investigated the presence of CD1d in tissues of the central nervous system (CNS).

METHODS: Archival formalin-fixed, paraffin-embedded MS and healthy control brain tissues were sectioned and then stained with luxol fast blue (LFB for myelin), and HLA-DR (class II MHC). Tissue blocks containing lesions were selected, and were categorized as either active, chronic active, or chronic silent based upon the HLA-DR and LFB staining characteristics. Sections were triple stained for CD1d, Iba-1 for microglia, glial fibrillary acidic protein (GFAP) for astrocytes, 4',6-diamidino-2-phenylindole (DAPI) for nuclei, and Sudan Black B for myelin and to minimize autofluorescence. Slides were imaged using an epifluorescent microscope, and lesions were outlined based on the presence or absence of myelin as shown by Sudan Black B. For chronic active lesions the edge was defined as a region extending up to 500 micrometers beyond the edge of complete demyelination. CD1d-positive cells were quantified per mm^2 and the numbers of cells double labeling for CD1d with Iba-1 or GFAP were noted.

RESULTS: CD1d-positive cells were present in MS tissue and absent in healthy tissue. CD1d positive cells were significantly more prevalent in areas of active demyelination in MS lesions, and colocalized primarily with GFAP-positive reactive astrocytes. The active edges of chronic active lesions contained CD1d-positive cells in similar numbers to active lesions, but had significantly more than the quiescent chronic active lesion centers. CD1d was also found occasionally within Iba-1 positive cells, however this occurred primarily in chronic silent lesions lacking active demyelination.

CONCLUSIONS: The finding of CD1d in the CNS of MS patients is novel and provide further support for a lipid-targeted autoimmune process contributing to the pathogenesis of MS. This lipid antigen presentation was especially high in areas of active demyelination, where CD1d is primarily expressed on GFAP-positive astrocytes. In contrast, there is an apparent transition to CD1d expression in Iba-1 positive cells in chronic silent lesions suggesting that the glia expressing CD1d may change over time.

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STEPHANIE SANTACRUZ

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GRANZYME B DISRUPTS EPITHELIAL BARRIER FUNCTION

Stephanie Santacruz[1,2,], Theo Klein[3], Leigh G. Parkinson[2,*], and David J. Granville[1,2,*]*

BACKGROUND/OBJECTIVES: Psoriasis, discoid lupus erythematosus, pressure ulcers, and periodontitis are a few of the many examples of chronic inflammatory diseases that result from a defective barrier function. A healthy epithelium functions as a barrier to the external environment, regulating temperature, water loss, and pathogen exposure. This barrier can be found in the skin, lung, and gastrointestinal tract and is maintained by intercellular junctions. Epithelial barrier disruption can result in increased infection, allergen exposure, and inflammation, culminating into severe constitutions. Granzyme B (GzmB) is a serine protease originally studied in immune cell-mediated apoptosis. However, recent work has found that GzmB is expressed by a variety of cells, can accumulate in the extracellular milieu, and is associated with aging, chronic inflammation and impaired tissue repair due to extracellular matrix (ECM) cleavage. As such, GzmB may contribute to the progressive loss of epithelial barrier function. We hypothesize that GzmB disrupts epithelial barrier function through the proteolytic cleavage of cell junction proteins.

METHODS: Human keratinocytes (HaCaTs) were treated with exogenous GzmB, in the presence or absence of a GzmB inhibitor. MTS assay was used to assess cell viability after GzmB treatment. Epithelial barrier function was assessed by measuring resistance across cell monolayers using Electric Cell-substrate Impedance Sensing (ECIS). E-cadherin was visualized on cell surface by confocal microscopy. Afterward, cell culture supernatants were assessed for presence of E-cadherin fragmentation by western blot analysis. Gingival crevicular fluids (GCF) were obtained from persons with varying severities of gingivitis (1-3) and periodontitis and levels of GzmB compared to healthy individuals using ELISA.

RESULTS: There was no significant difference in cell viability between the GzmB treated/ untreated groups. GzmB treatment resulted in a loss of E-cadherin staining on the cell membrane which was further supported by western blot analysis of the cell supernatants, where we observed a dose-dependent increase in E-cadherin fragmentation. Confluent monolayers of HaCaT cells measured by ECIS show a marked decrease in resistance (barrier function) when treated with GzmB while cells treated with GzmB and inhibitor remained unaffected. GzmB levels in the GCF samples showed a significant difference between healthy controls and periodontitis ($p=0.0094$) and gingivitis 3 ($p=0.0493$), with gingivitis 1 & 2 showing no significant difference.

CONCLUSIONS: In summary, GzmB contributes to a decline in epithelial barrier function in part through the proteolytic cleavage of E-cadherin. Discovering how GzmB prevents proper wound healing could lead to the development of topical inhibitors that will aid in treatment of chronic skin wounds commonly seen in diabetic foot ulcers, and autoimmune disorders.

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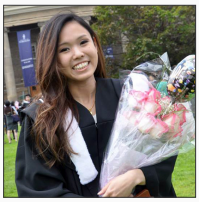

NICHA BOONPATTRAWONG

- *GRADUATE STUDENT*
- *ORAL SESSION*
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MATERNAL OBESITY AND EXERCISE PROGRAMS GLUCOSE HOMEOSTASIS AND CARDIOVASCULAR HEALTH IN OFFSPRING

Nicha Boonpattrawong[1], Daven Tai[2], Rika Aleliunas[2], Elianne Abramovich[2], Ismail Laher[3], Angela Devlin[1,2]

BACKGROUND/OBJECTIVES: The theory of developmental programming suggests that fetal and early postnatal environment can influence risk for chronic diseases, such as cardiovascular disease (CVD), later in life. Approximately 50% of women of childbearing age are overweight (BMI 25-29.9 kg/m2) or obese (BMI ≥ 30 kg/m2) in Canada. Several rodent studies have reported that offspring from obese dams have greater adiposity, insulin resistance, and vascular endothelial dysfunction – an early indicator of CVD. Vascular endothelial dysfunction is characterized by decreased bioavailability of nitric oxide. Exercise been shown to increase availability of nitric oxide should improve vascular endothelial function and also improves insulin sensitivity. We hypothesize that maternal exercise will mitigate the adverse effects of maternal obesity during pregnancy on vascular dysfunction and insulin resistance in offspring.

METHODS: Female (C57BL/6) mice were fed from weaning a control diet (10% kcal fat) or western diet (45% kcal fat) to induce excess adiposity. At 13 weeks of age, female mice were put into cages with or without access to a running wheel for voluntary exercise throughout breeding, pregnancy and lactation. Male offspring were weaned onto a control or western diet. At 13 weeks post weaning, glucose homeostasis was assessed by intraperitoneal glucose (IPGTT) and insulin tolerance test (IPITT). Vascular endothelial function (ex vivo) was assessed in aortic rings by isometric force measurement for vasoconstriction and vasodilatation. Serum insulin concentrations were quantified by ELISA. Slc2a4 (encodes GLUT4) and Ppargc1a (encodes PGC1α) mRNA expression in skeletal muscle was quantified by Real-Time PCR using the ΔΔCt method of relative quantification.

RESULTS: At 13 weeks on the post weaning diet, western-fed offspring from western diet-fed dams without exercise (MWS) had greater glucose intolerance (IPGTT AUC; $p<0.05$) than those from exercised dams (MWE). No differences were found in insulin tolerance in response to IPITT or serum insulin concentrations. Vascular function assessments showed that MWE offspring had greater sensitivity (EC50; $p<0.05$) and greater maximum percent vasodilatation ($p<0.05$) induced by acetylcholine than MWS offspring. No significant difference was observed in SNP-induced vasodilatation. These effects on glucose homeostasis and vascular function were not observed in offspring fed the post-weaning control diet. Western diet-fed offspring from exercised dams had higher Ppargc1a ($p=0.077$) and Slc2a4 ($p=0.039$) mRNA expression
in skeletal muscle than those from sedentary dams.

CONCLUSIONS: Offspring from obese dams that exercise during pregnancy have improved glucose tolerance and vascular function in adulthood. The findings suggest that maternal exercise during pregnancy improves cardiometabolic health of the offspring. These results infer beneficial effects of exercise during pregnancy in women with obesity and can be useful in setting guidelines for exercise during pregnancy.

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JON OBST

- *GRADUATE STUDENT*
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- *SUPERVISOR: DR. MARIANNE SADAR*

CHRONIC EXPOSURE TO A NOVEL ANDROGEN RECEPTOR N-TERMINAL DOMAIN INHIBITOR INDUCES RESISTANCE VIA A SELECTIVE METABOLISM PATHWAY

Jon Obst[1], Jun Wang[1], David Williams[2], Amy Tien[1], Nasrin Mawji[1], Yu Chi Yang[1], Raymond Andersen[2], Marianne Sadar[1]

BACKGROUND/OBJECTIVES: The androgen receptor (AR) has long been recognized as playing a crucial role in prostate cancer (PCa) maintenance and progression; therefore its inhibition has been the cornerstone of modern therapy for men who fail primary treatment. Current treatments are initially effective, however resistance ultimately develops and the disease progresses to a lethal form termed metastatic castration-resistant prostate cancer (mCRPC). 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 machinery, and is vital for a transcriptionally functional receptor. The lead compound, EPI-002 (ralaniten/RAL) specifically inhibits both full-length AR and constitutively active AR splice variants. The efficacy of ralaniten-acetate, the prodrug of ralaniten, is currently being tested in a Phase I/II clinical trial for mCRPC (NCT02606123). Here we propose a novel resistance mechanism arising from sustained AR-NTD inhibition, with the goal of pre-emptively developing backup compounds.

METHODS: The androgen sensitive human PCa cell line LNCaP was used in all experiments. A resistant cell line (LNCaP-RALR) was generated by passaging parental LNCaP cells weekly in media supplemented with RAL beginning in September 2012. ***In vitro*** and in vivo studies using LNCaP and LNCaP-RAL^R cells were employed to confirm biological resistance. A human affymetrix microarray identified increased expression of UGT2B genes involved in drug metabolism in the resistant line and was validated using qRT-PCR, western blot and functional studies. LNCaP and LNCaP-RAL^R cells were challenged with EPI-045, an EPI-analog predicted to remain effective in the context of RAL resistance. Sensitivity to EPI-045 was confirmed using LNCaP-RAL^R xenografts.

RESULTS: LNCaP-RALR cells treated with 25 μM RAL displayed similar growth rates to vehicle treatment, both in vitro and in vivo. Conversely, parental LNCaP cells showed significant growth inhibition in response to RAL treatment. LNCaP-RALR cells retained sensitivity to anti-androgens and AR knock-down by targeted siRNA, implying functional AR remains essential for growth. qRT-PCR data indicated that RAL had reduced ability to block AR mediated gene transcription. Interrogation of microarray data revealed candidate genes (UGT2B family) which were specifically upregulated in the resistant line, and may function to metabolize RAL. As expected, UGT2B activity was also demonstrated to be enhanced in LNCaP-RAL^R cells compared the control line. Supporting this hypothesis EPI-045, which is predicted to be resistant to UGT2B metabolism, was able to significantly inhibit LNCaP-RAL^R proliferation and AR transcriptional activity - both in vitro and ***in vivo***.

CONCLUSIONS: Taken together these data suggest resistance to RAL is mediated by a mechanism involving preferential metabolism by UGT2B genes. LNCaP-RAL^R cells remain dependent upon AR signalling, and are sensitive to anti-androgens used clinically as well as a novel EPI-analog. This work highlights the potential for combination or sequential therapy in the context of drug-resistant mCRPC.

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WENCHEN ZHAO

- *GRADUATE STUDENT*
- *ORAL SESSION*
- *SUPERVISOR: DR. CHERYL WELLINGTON*

NOVEL PURINERGIC RECEPTOR 7 (P2X7) ANTAGONISTS UPREGULATE APOLIPOPROTEIN E IN HUMAN ASTROCYTES

Jianjia Fan[1], Rui Qi Zhao[1], Cameron Parro[1], Wenchen Zhao[1], Tarek Deeb[2], John Linley[3], Michael Wood[4], Iva Kulic[1], Cheryl Wellington[1*] * Co-senior authors*

BACKGROUND/OBJECTIVES: Alzheimer's disease (AD), the cause of 60% to 70% of overall dementia, is a chronic neurodegenerative disease. One of the hallmarks of AD is the accumulation of amyloid plaques in the brain. Apolipoprotein E (apoE), which carries lipids in the brain in the form of lipoproteins and is transcriptionally regulated by the Liver X Receptor (LXR) nuclear receptor, plays an undisputed role in AD pathophysiology. The APOE gene is the most highly associated susceptibility locus for late onset AD, and has well-established associations with amyloid deposition and clearance from the brain. We and others have shown that lipidation of apoE can assist amyloid clearance, raising interest in augmenting apoE function as a proposed therapeutic strategy for AD. Manipulating apoE levels and lipidation is of interest to better understand apoE functions in brain aging and AD. The objective of this project is to characterize a new class of small molecule compounds, purinergic receptor P2X7 antagonists, as apoE modulators. We hypothesize that P2X7 regulates expression and secretion of apoE in human astrocytes.

METHODS: A high-throughput phenotypic screen was conducted using the CCF-STTG1 human astrocytoma cell line to identify small molecules that could upregulate apoE secretion. To validate P2X7 antagonists, identified as hits in the screen, a P2X7-/- CCF-STTG1 cell line was generated and its responsiveness to P2X7 antagonists was analyzed. To determine if the LXR activity is required in apoE upregulation mediated by P2X7 antagonists, CCF-STTG1 cells were treated with LXR antagonist, GSK2033, to abolish LXR activity. LXR knock-out (KO) MEF cells were also treated with P2X7 antagonists and apoE mRNA level was determined by RT-qPCR.

RESULTS: P2X7 antagonists were validated as inducers of apoE expression and secretion in CCF-STTG1 cells as the P2X7-/- CCF-STTG1 cell line lost apoE upregulation following treatment with P2X7 antagonists. In both GSK2033-treated CCF cells and LXR KO MEF cells, P2X7 antagonists failed to upregulate apoE expression, indicating indirect involvement of the LXR pathway in apoE regulation.

CONCLUSIONS: Here we characterize P2X7 antagonists as apoE modulators in CCF-STTG1 cells, which are able to upregulate apoE only in parental but not in P2X7-/- CCF-STTG1 cells. Experiments employing pharmacological and genetic suppression of LXR activation suggest that these P2X7 antagonists require functional LXR to induce apoE secretion, even though they are not direct LXR ligands. These findings reveal a novel link between the P2X7 receptor and the apoE/LXR pathway.

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MARYAM GHAEDI

- *GRADUATE STUDENT*
- *ORAL SESSION*
- *SUPERVISOR: DR. FUMIO TAKEI*

ANALYSIS OF INNATE LYMPHOID CELL SUBSETS SUBSETS IN ALLERGIC LUNG INFLAMMATION

Maryam Ghaedi[1,2] and Fumio Takei[1,2]

BACKGROUND/OBJECTIVES: Innate lymphoid cells (ILCs) are divided into group 1 (ILC1), group 2 (ILC2) and group 3 (ILC3). ILCs share a common precursor, ILC progenitor (ILCP), which expresses the transcription factor RORα. Allergic lung inflammation is driven by overproduction of type 2 cytokines, including IL-5, and IL-13. Upon allergic encounter, a damaged lung epithelium releases alarmin IL-33 and activates ILC2s. Activated ILC2s produce large amounts of IL-5 and IL-13, which induce type 2 inflammation. As chronic type 2 inflammation can cause asthma, ILC2s play a critical role in asthma development. Recently, a new subset of ILC2s, termed inflammatory ILC2, has been found. Inflammatory ILC2s are stimulated by alarmin IL-25 and produce IL-5 and IL-13. Upon lung infection with Candida albicans these cells produce IL-17, which is known to be involved in asthma. ILC2s or a subset of these cells can also trans-differentiate into interferon-γ producing ILC1s. Thus, allergic lung inflammation seems to be regulated by complex interactions of multiple ILC subsets and their plasticity. Our objective is to identify and characterize ILCs, which regulate allergic lung inflammation.

METHODS: To identify and characterize ILCs, which regulate allergic lung inflammation, I crossed RORα-IRES-Cre knock-in mice with Rosa26-^{flox}STOP^{flox}-YFP mice to generate RORα/YFP mice, in which cells expressing RORα during development are permanently marked by YFP. RORα is expressed in ILCP. Hence, all ILCs are labeled by YFP.

RESULTS: My preliminary analysis of these mice has shown that lung lineage-negative YFP$^+$ cells include not only ILC2s (Thy1$^+$CD127$^+$CD25$^+$ST2$^+$) but also Thy1$^+$CD127$^+$CD25$^-$ST2$^+$ and CD25$^+$ST2$^-$ cells, which are different from known ILCs. These cells produce IL-5 and IL-13 upon IL-33 stimulation. Interestingly, the response of these subsets is different from each other upon stimulation with bacterial derived lipopolysaccharide.

CONCLUSIONS: These results provide new insights into ILC heterogeneity and their contribution to allergic lung inflammation development.

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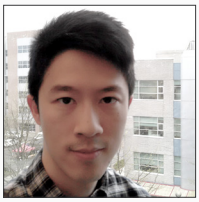

BRYAN LIN

- *GRADUATE STUDENT*
- *ORAL SESSION*
- *SUPERVISOR: DR. ED PRYZDIAL*

HOST COAGULATION INITIATED ON THE VIRUS SURFACE

Bryan Lin[2,3], Michael Sutherland[1,2,3] and Edward Pryzdial[1,2,3]

BACKGROUND/OBJECTIVES: News reports of dengue (DENV), Zika, chikungunya, and Ebola infections are reminders that viruses are a prevalent threat to both healthcare and blood collection systems. To address the lack of defences against these and other emerging pathogens, we hypothesized that many viruses have common host-derived constituents within the surrounding membrane bilayer, termed envelope. To explain numerous virus-hemostatic disease links, we previously showed that the envelope of three herpesviruses acquire tissue factor (TF) and anionic phospholipids (aPL) from the host, both vital in physiological blood coagulation. Viral TF and aPL accelerate the activation of clotting factor (F) X to FXa by FVIIa, leading to clotting and cell signaling. Using herpes simplex virus type 1 (HSV1) as a model virus, our lab has shown that both ***in vitro*** and ***in vivo*** infection is inhibited when the virus lacks TF. Furthermore, HSV1 has evolved to modulate TF function through virus-encoded glycoprotein C (gC). Our current objectives are to: a) determine if other enveloped viruses obtain TF and/or aPL; and b) dissect the involvement of viral TF/gC on virus-mediated clotting.

METHODS: TF^{+}/TF^{-} HSV1 variants and DENV propagated in cell cultures were purified and characterized. Immunogold electron microscopy was used to simultaneously visualize TF, aPL and a virus-encoded marker on the virus surface. Plasma clotting induced by HSV1 was characterized in human plasma. FX activation by virus and the effect of solubilized gC (sgC) was followed in a FXa chromogenic assay.

RESULTS: Individual HSV1 and DENV particles incorporated TF and aPL into their envelope. Viral TF and gC were required for optimal FX activation, and TF was essential for gC-mediated enhancement. This was confirmed using purified membrane-bound TF, in the presence or absence of sgC. In plasma, gC enhanced TF-dependent clotting induced by HSV1, which was mitigated by an alternative FX-activating clotting pathway.

CONCLUSIONS: For the first time, enveloped viruses from distinct families are shown to acquire the host coagulation initiating factors, TF and aPL, supporting the idea that targeting viral TF may facilitate broad-spectrum anti-viral development. Virus surface TF and aPL may explain the correlations to a variety of hemostatic imbalances.

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ALBERTO DELAIDELLI

- *GRADUATE STUDENT*
- *ORAL SESSION*
- *SUPERVISOR: DR. POUL SORENSEN*

TARGETING TRANSLATION ELONGATION IN PEDIATRIC NEUROBLASTOMA

Alberto Delaidelli[1,2], Gian Luca Negri[1], Asad Jan[1,2], Brandon Jansonius[1], Debjit Khan[1,3], Gabriel Leprivier[1,4], Marcel Kool[5,6], Stefan M Pfister[5,6], Marc Remke[7], Michael D Taylor[7], John M Maris[8] and Poul H Sorensen[1,2]

BACKGROUND/OBJECTIVES: Every day, 43 children in North America are diagnosed with cancer, the number one cause of death by disease for children in the Western world. Neuroblastoma (NB) is the most common pediatric extracranial solid cancer, causing ~15% of all childhood related cancer deaths. Hence, there is a dire need to identify and validate novel and more effective therapeutic approaches. Tumors are continually exposed to acute changes in the micro-environment, including limited nutrient availability. We previously showed that eukaryotic Elongation Factor-2 Kinase (eEF2K) is a critical regulator of cellular adaptation to acute nutrient deprivation. Our preliminary data indicate that NB cells are particularly resistant to nutrient deprivation and express a very high level of eEF2K. In addition, high mRNA eEF2K expression is constantly associated with poor prognosis in cancer, including NB. Based on those findings, we hypothesized that eEF2K mediates NB adaptation to acute metabolic stress, and that inhibiting this pathway can impair NB progression.

METHODS: Initially, immunohistochemistry for key molecules in the pathway (p-eEF2 Thr56) was performed on NB tissue microarrays (TMAs) to link results with clinical outcome. Effects of eEF2K genetic inactivation on cell survival were evaluated ***in vitro*** in NB cell lines under nutrient deprivation. Cell viability and cellular apoptosis were assessed by MTT assay, PI staining and western blotting for cleaved caspase 3. Finally, NB xenografts in mice, either fed ad ***libitum*** or kept under caloric restriction, were used to confirm ***in vitro*** observations.

RESULTS: Low eEF2K activity is predictive of improved survival in NB. In vitro inactivation of eEF2K markedly decreases survival of NB cell lines under nutrient deprivation. Growth of NB xenografts is markedly impaired by eEF2K knockdown, particularly when mice are fed with caloric restricted diet.

CONCLUSIONS: eEF2K represents a critical mechanism for NB adaptation to acute metabolic stress, and is therefore a promising therapeutic target. We are currently exploring the pharmacological inhibition of eEF2K in xenograft tumor models. Future therapeutic studies will aim to combine eEF2K inhibition with caloric restriction mimetics such as metformin or glycolysis inhibitors, as eEF2K activity appears to be critical under metabolic stress conditions.

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CODY LO

- *SUMMER STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. MARCEL BALLY*

COPPER DRUG COMPLEXES (CDCS) AS A NOVEL TREATMENT OPTION FOR PLATINUM RESISTANT CANCERS

Cody Lo, Mohamed Wehbe, Ada Leung, Marcel Bally

BACKGROUND/OBJECTIVES: Platinum drugs are the most successful inorganic medicinal compounds and are involved in approximately 50% of chemotherapeutic regimens. However, drug toxicity and resistance remain to be clinical challenges. Thus, identifying and developing new therapeutic entities to treat cancer is of high importance. Copper has been the focus of medicinal inorganic chemistry programs as a viable alternative to platinum owing to the natural pathways the human body has to detoxify copper. We selected five bidentate copper ligands (Diethyldithiocarbamate (DDC), Pyrithione (Pyr), Plumbagin (Plum), 8-hydroquinoline (8-HQ), and Clioquinol (CQ)) known to form dimeric copper drug complexes (CDCs). We tested their cytotoxicity in a panel of 8 cancer cell lines that are known to be treated with Platinum and have varying degrees of sensitivity.

METHODS: We conducted drug assays to determine the cytotoxicity of both the ligands and their CDCs. Prior to drug treatment, the cells were seeded into 384 well plates and allowed to grow for 24 hrs. The adherent cell lines (A549, A2780-S, A2780-CP, FaDu, Cal-27, SCC-25, H1933) were exposed to the indicated compounds for 72 hrs and then stained with Hoescht 33342 and ethidium homodimer I for total and dead cell counts, respectively. Cells were imaged using the InCELL Analyzer 2200 and cell viability was measured based on viable nuclei count. The suspension cell line (MV-4-11) was also exposed for 72 hrs but was instead analyzed using PrestoblueTM, a cell viability assay based on metabolism. Assays were also conducted using two common platinum drugs (Cisplatin and Carboplatin) and with copper sulfate as controls. DDC, CQ, 8-HQ, Plum and their corresponding copper complexes were solubilized in DMSO and cells were dosed such that the final DMSO concentration was 0.05%. Pyr was dissolved in sterile water.

RESULTS: Cell lines demonstrated marked differences in platinum sensitivity. For example, the IC50 value of Cisplatin and Carboplatin in the A2780-S cell line increases by 3.7 and 8.5-fold when used to treat A2780-CP cells; an isogenic platinum resistant cell line. Plum and 8-HQ were found to be the most active ligands whereas DDC and CQ were the least active. $Cu(DDC)_2$ and $Cu(Pyr)_2$ were the most active CDCs in the panel and markedly higher than their corresponding ligands by themselves. $Cu(Plum)_2$ and $Cu(CQ)_2$ also showed modest improvements in activity compared to their non-copper bound form, but the activity of $Cu(CQ)_2$ would not be considered therapeutically relevant. In general, the ligands appeared to be less active in the platinum resistant cell lines whereas the CDCs maintained their higher activity regardless of platinum sensitivity.

CONCLUSIONS: In conclusion, this study found that the cytotoxicity of many CDCs exceeded that of known platinum based compounds and demonstrate CDCs as a potential new class of therapeutics in cancer. We screened 5 ligands that have reported CDCs with anti-cancer activity and found that 4 showed cytotoxicity below 10 μM in all 8 cancer cell lines screened.

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URINARY TRACT INFECTION IN PREMATURE NEONATES: A -5YEAR RETROSPECTIVE STUDY

King Mong Tong[1], Ghada Al-Rawahi[2], Joseph Ting[3], David Goldfarb[3] , Vanessa Paquette[4], Kristopher Kang[3], Horacio Osiovich[3], Ashley Roberts[3]

BACKGROUND/OBJECTIVES: Urinary tract infection (UTI) is a common cause of morbidity in infants and children. In the neonatal population there is no clear criterion for diagnosis of UTI. Laboratory findings and clinical presentations in neonates could be different from older children. Clinical presentation of infants with UTI can be non-specific and delayed diagnosis or inadequate treatment may lead to prolonged hospitalization and renal complications. There is a paucity of data available on the prevalence, microbiology, concomitant morbidities, and associated urological abnormalities exclusively in preterm infants.

The objectives are to determine the incidence, microbiology, and co-morbidities in a cohort of preterm infants (<37 weeks) with UTI admitted to a tertiary perinatal center.

METHODS: A retrospective study of all urine culture samples collected from preterm infants during January 2010 to December 2014 was conducted. Urine culture results were classified as definite UTI (isolation of a single uropathogen from catheterized urine sample with growth ≥ 10^5 colony forming units (CFU)/ml) and probable UTI (growth between 10^4 and 10^5 CFU/mL). Contaminant (mixed organisms, growth < 10^4 CFU/ml, or growth of non-uropathogens) samples were not included in our analysis.

RESULTS: There were 45 episodes of UTI (17 definite & 28 probable) among 39 patients during the study period, with an overall incidence of 2.3% among all NICU admissions. Median gestational age was 27 weeks (IQR=25-32) and median birth weight was 1017g (IQR=718-1583). The median day of life at the onset of UTI was 38 days (IQR=25-88). Five (11%) patients had concomitant invasive infection (3 bacteremia and 2 meningitis). Thirty UTI episodes had urinalysis performed, with 11 (36.7%) and 14 (46.7%) being positive for leucocyte esterase and nitrites, respectively. Among the 36 infants who had a kidney ultrasound performed, 7 (19.4%) neonates had anomalies identified, namely hydronephrosis, multicystic dysplastic kidneys, and hydroureteronephrosis. The most prevalent organisms isolated were Enterococcus faecalis (n=12, 27%), Enterobacter species (n=11, 24%) and Escherichia coli (n=7, 16%). All E. faecalis were susceptible to ampicillin and 59% of Enterobacteriaceae isolated were resistant to cefotaxime. There was no significant difference in gestational age, birth weight, and day of life at UTI onset between infants with definite vs probable UTI and cefotaxime resistant vs susceptible gram negative cultures.

CONCLUSIONS: The incidence of UTI in preterm infants in a tertiary perinatal centre was 2.3%, but 59% of Enterobacteriaceae strains were resistant to cefotaxime.

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ASSOCIATION BETWEEN ETHNICITY AND CARDIOVASCULAR DISEASE WITH ELEVATED LIPOPROTEIN (A)

Kurt Vandevoorde[1], Jiri Frolich[2], Ramesh Saeedi[3]

BACKGROUND/OBJECTIVES: Elevated lipoprotein(a) [Lp(a)] is an independent risk factor for cardiovascular disease (CVD). Race-based differences in median Lp(a) have been documented but it is unknown that ethnicity may modify Lp(a) and risk of CVD. Thus, in the current study determine the prevalence of CVD among individuals of different ethnicities living with elevated Lp(a).

METHODS: This is a retrospective chart review of adults seen at the lipid clinic at St. Paul's Hospital whom their Lp(a) is measured. Demographic information as well as clinical information and lipid profile are recorded. CVD event is defined as having stenosis, myocardial infarction, vascular disease, and/or coronary bypass graft surgery.

RESULTS: So far 59 patients are identified having their Lp(a) is measured with median age of 44 years (29-69). The median and mean Lp(a) levels among different ethnicities are summarized in Table 1. 2 (15%) of the Middle Eastern, 1 (20 %) of each South Asian and Southern Asian and 9 (28%) of European patients has CVD events.

CONCLUSIONS: This study will allow us to assess ethnicity as a potential factor related to high Lp(a) and increased risk of CVD. Also it illustrates whether certain ethnicities may be protected from the pathogenic influence of Lp(a).

Statistic¤	n¤	Minimum¤	Maximum¤	Median¤	1st· Quartile¤	3rd· Quartile¤	Mean¤
Middle·Eastern¤	13¤	20¤	771¤	157¤	33¤	230¤	205.6¤
South·Asian·¤	5¤	42¤	295¤	83¤	68¤	117¤	121¤
European¤	32¤	7¤	1700¤	120¤	68¤	293¤	301¤
Southern·Asian¤	5¤	136¤	690¤	344¤	180¤	348¤	340¤
Latin·American¤	1¤	255¤	255¤	255¤	255¤	255¤	255¤
African¤	2¤	348¤	398¤	373¤	361¤	386¤	373¤
East·Asian¤	1¤	1700¤	1700¤	1700¤	1700¤	1700¤	1700¤

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BRONCHODILATORY EFFECT OF DEEP INSPIRATION IN FRESHLY ISOLATED SHEEP LUNGS

William Wong[3], Lu Wang[1,3], Peter Paré[1,3] and Chun Seow[2,3]

BACKGROUND/OBJECTIVES: Taking a big breath is known to reverse bronchoconstriction induced by bronchochallenge in healthy subjects; this bronchodilatory effect of deep inspiration (DI) is diminished in asthmatics. The mechanism underlying the DI effect is not clear. Observations from experiments using isolated airway smooth muscle (ASM) preparations and airway segments suggest that straining of ASM due to DI could lead to bronchodilation, possibly due to strain-induced reduction in ASM contractility. However, factors external to the lung cannot be excluded as potential causes for the DI effect. Neural reflex initiated by stretch receptors in the lung are known to inhibit the broncho-motor tone and enhance vasodilatation; the former directly reduces airway resistance, and the latter facilitates removal of contractile agonists through the bronchial circulation. If the DI effect is solely mediated by factors extrinsic to the lung, the DI effect would be absent in isolated, nonperfused lungs. Here we examined the DI effect in freshly isolated, nonperfused sheep lungs in order to determine whether intrinsic properties of the lung contribute significantly to bronchodilation.

METHODS: Lungs from adult sheep were obtained from a local abattoir. Lungs were connected to a custom-build plethysmograph and transpulmonary pressure was measured across a Fleish pneumotachograph. Transpulmonary pressure was measured over the respiratory cycle and used to calculate pulmonary resistance. To determine the effect of lung volume on pulmonary resistance and be able to correct for the volume effect in data analysis, pulmonary resistance was measured while the lung was ventilated at different transpulmonary pressures (and thus, different volumes). For determining the effect of DI on resistance: DI (corresponding to a transpulmonary pressure of -40 cmH2O), lasting for 1 breathing cycle, was applied to the unchallenged lung (control). The lung was then exposed to nebulized acetylcholine or histamine solutions of increasing concentration until the peak-to-peak flow decreased by 40-50%. Lastly, a second DI was applied to the challenged lung. Pulmonary resistance was was measured before and after DIs to determine the presence or absence of a bronchodilatory effect.

RESULTS: The imposition of DI on isolated lungs resulted in significant bronchodilation and this DI effect was present only after the lungs were challenged with a contractile agonist (acetylcholine or histamine). There was no significant change in pulmonary resistance with DI in unchallenged lungs ($p = 0.443$). After DI with acetycholine challenge, pulmonary resistance dropped from 25.26 ± 5.05 cmH2O•s/L to 13.82 ± 4.49 cmH2O•s/L, which was a significant decrease ($p < 0.01$). DI after histamine challenge also produced a statistically significant drop in pulmonary resistance ($p < 0.05$).

CONCLUSIONS: In the absence of neural reflexes, a strong DI-induced bronchodilatory effect is still present in isolated sheep lungs. Therefore, a significant portion of the bronchodilatory DI effect must stem from factors internal to the lung, likely related to the activation of ASM.

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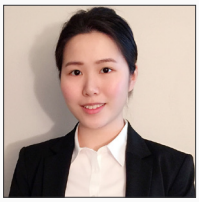

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EXPLORATION OF NRG1 FUSIONS AND OTHER GENOMIC DRIVERS IN CHOLANGIOCARCINOMA

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BACKGROUND/OBJECTIVES: Cholangiocarcinoma (CHOL) is an aggressive cancer with 5-year survival of 2-30% depending on location and staging[1]. Current standard of care requires complete surgical removal of all tumours1. Despite being a rare neoplasm with an incidence of 1-2 cases per 100,000, the incidence of CHOL is on the rise1. We had previously identified, for the first time, a gene fusion event involving NRG1 in a patient with primary intrahepatic cholangiocarcinoma[2]. NRG1 fusion is more prominent as a genomic cancer driver in the mucinous subtype of lung adenocarcinoma[2]. Importantly, the NRG1 fusion protein is a known molecular target of afatinib, a HER- family kinase inhibitor[2]. This patient received afatinib with dramatic clinical response[2]. Finding a similar gene fusion in other patients with CHOL could justify routine screening of future patients for targeted pharmacologic treatment.

METHODS: In this study, Vancouver General Hospital patient cases with intrahepatic and extrahepatic CHOL were evaluated for presence of NRG1 fusion. We identified 52 CHOL patients from year 2012-2016 and reviewed the pathology, and a tissue microarray (TMA) was constructed using archival formalin-fixed paraffin-embedded tissue from these cases. The TMA was screened using a fluorescent in situ hybridization (FISH) assay for detection of NRG1 rearrangement that we developed and validated. In addition, we extracted DNA from these samples, and used a validated genotyping assay to interrogate for hotspot mutations in codon 132 of IDH1, which has been reported in 10-15% of cases. This mutation has been implicated in the pathogenesis of some cancers and maybe a predictor of response to IDH- inhibitors[2].

RESULTS: Although we confirmed the presence of NRG1 rearrangement in the fusion-positive index case in our TMA, we were not able to identify additional cases with NRG1 fusion in this initial cohort of 52 patients. However, we were able to confirm hotspot mutations in IDH1 in 13.4% of cases which is comparable to the data generated by The Cancer Genome Atlas (TCGA).

CONCLUSIONS: While the prevalence of NRG1 fusion in CHOL cases may be rare, more patient samples should be evaluated since the presence of this targetable fusion has significant therapeutic implications. We plan to expand this study by exploring a larger case series of CHOL from multiple institutions.

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References:
Nakamura, Hiromi et al (2015). Genomic spectra of biliary tract cancer. Nature Genet. 47 (9) 1003-1013
Jones, Martin et al (2017). Successful targeting of the NRG1 pathway indicates novel treatment strategy for metastatic cancer. Annals of Oncology, in revision.

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INCREASED BLOOD MITOCHONDRIAL DNA MUTATIONS IS ASSOCIATED WITH OLDER AGE, AND PEAK HIV VIRAL LOAD, BUT NOT HIV STATUS

Adam Ziada[1,2], Meng Ying Lu[1,2], Jarek Ignas-Menzies[1,2], Sara Saberi[1,2], Beheroze Sattha[1,2], Hélène Côté[1,2,3] and the CIHR team grant on cellular aging and HIV comorbidities in women and children (CARMA)

BACKGROUND/OBJECTIVES: People living with HIV appear to experience accelerated aging. The accumulation of somatic point mutations is believed to be a marker of biological aging, and has been implicated in many age-associated diseases that manifest prematurely in HIV+ individuals. We hypothesized that mtDNA somatic substitutions would increase with older age and HIV infection.

METHODS: Participants in this cross-sectional study were HIV+ (n=92, 12 <19y) and HIV- (n=72, 13<19y) females enrolled in the CARMA cohort, not infected with hepatitis C or B virus, and either current or never (but not past) smokers. Whole blood DNA was extracted and somatic mtDNA substitution mutation rates/10,000bp were quantified via next generation sequencing with primer IDs. Factors associated with mtDNA mutations were investigated through Spearman's correlations, Mann-Whitney tests, and ANCOVA of log10 (x+1) values.

RESULTS: Measured blood mtDNA mutation rates met quality control for 75 HIV+ and 63 HIV- individuals (median [IQR] (range) of 0.5 [0.3-0.6] (0.0-1.64)) aged 1-75 years. Ages were similar between the two groups (p=0.18) and all HIV+ children had undetectable pVL while this was true for 59% of adults, 32% of which were current smokers. A significant correlation was seen between mtDNA mutation rates and age (rho=0.36, p<0.001) but there was no association with HIV+ status (p=0.49), among all participants, or smoking (current vs. never, p=0.76) in adults. Among HIV+ adults, higher mtDNA mutation rates were associated with peak HIV pVL recorded (> vs. ≤100,000 copies/mL, p=0.018) but not current HIV pVL (detectable vs non-detectable) (p=0.69), CD4+ count (rho=-0.14, p=0.28), or CD4 nadir (rho=-0.21, p=0.10). In a multivariable model of HIV+ adult participants (R^2=0.16) that included age (p=0.05) and peak pVL (p=0.03), both remained independently associated with mtDNA mutations rates.

CONCLUSIONS: Somatic mtDNA mutations can be measured in blood and their rates increase with age, and peak HIV viral load.

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EXTINCTION KINETICS OF POST-PHLEBOTOMY CIRCULATING DNA EXTRACTED FROM PATIENT BLOODS STORED IN STRECK© CELL-FREE BLOOD COLLECTION TUBES

Brian Yu Chieh Cheng[1], Damian Yap[2], Stephen Chia[3], Samuel Aparicio[4]

BACKGROUND/OBJECTIVES: It has been shown that tumour cells release circulating tumour DNA (ctDNA) into blood circulation during disease progression, which can be detected via next-generation sequencing or digital PCR. However, the process of extracting ctDNA involves post-phlebotomy isolation of blood plasma, which is conventionally prone to genomic DNA contamination via lymphocyte lysis. This contamination increases the difficulty of detecting ctDNA, thus it is customary practice when dealing with patient plasmas to minimize such contamination by processing blood samples within 2 hours. A recently developed blood collection tube called the Streck blood collection tubes (BCT) has been shown to prevent patient lymphocyte lysis for up to 14 days; a drastic improvement over the traditional 2-hour time-limit placed on plasma isolation. We aim to monitor the extinction kinetics of genomic DNA contamination in plasma extracted from bloods stored in BCTs for up to 10 days, and streamline methodologies to detect ctDNA in non-metastatic breast cancer patients.

METHODS: Twenty breast cancer patients recruited in the Biology of Breast Cancer Clinical Research Project were selected to participate in this study. Per patient, blood plasmas were collected in the novel BCTs and traditional blood collection tubes (EDTA tubes). Blood plasma and circulating DNA was then subsequently isolated 2 hours, 7 days, and 10 days' post-phlebotomy. Patient tumour DNA was subsequently extracted from tumour blocks, and sequenced using Illumina's TruSeq Cancer Panel to discover a set of tumour-specific mutations to serve as digital PCR targets. Custom probe-based PCR assays were then developed to detect these patient-tumour-specific mutations in their blood plasma using digital droplet PCR (ddPCR).

RESULTS: Plasma derived circulating DNA have been extracted for all patients (n = 20); however only 12 of them have had surgery that generated sufficient amounts of tissue that can be used for DNA sequencing. Of the 12 patients sequenced using the TruSeq Cancer Panel, 8 patients showed true tumour mutations that were deemed valid to be used as targets for ddPCR. Currently, we have 7 custom assays designed for ddPCR, and are in the process of optimizing these assays to validate the tumour specific mutations as ctDNA in patient plasmas.

CONCLUSIONS: Currently, BCT's seemed capable of preventing major lymphocyte lysis for up to 10 days based on initial circulating DNA concentrations when compared to plasmas isolated 2 hours after phlebotomy. Using a strand specific oligo protocol during sequencing library construction, we were able to discover 8 patient specific breast cancer mutations that were likely not due to sequencing or tissue fixation artifacts. Next steps in our project will involve detection of these mutations in plasma derived circulating DNA using custom-designed assays via ddPCR.

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IDENTIFICATION OF NOVEL MARKERS IN ENDOMETRIAL ADENOCARCINOMA MOLECULAR SUBGROUPS, USING PROTEOMICS AND IMMUNOHISTOCHEMISTRY

Kendall Greening[1], Basile Tessier-Cloutier[2], Dawn Cochrane[3], Lien Hoang[2], Jessica N McAlpine[3]

BACKGROUND/OBJECTIVES: Endometrial adenocarcinomas (EC) encompass a variety of histologic types with differing prognoses and management. Clinicopathologic classification alone has been shown to be suboptimal in separating EC into clinically relevant prognostic groups, driving the move towards a molecular based classification scheme. Based on data from The Cancer Genome Atlas (TCGA), our group developed a pragmatic and prognostically-relevant molecular classification tool for EC (ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer) which separates EC into 4 distinct prognostic groups: 1) MMR-D (mismatch repair deficient), 2) POLE EDM (polymerase-ε exonuclease domain mutated), 3) p53 wt (p53 wild-type) and 4) p53 abn (p53 mutated). In this study, we attempt to identify additional biomarkers within each of the EC molecular groups using mass spectrometry based proteomics, and subsequently test immunohistochemistry (IHC) on selected proteins that may be of use for future clinical purposes.

METHODS: Full proteome analysis of 52 cases of EC, previously stratified by ProMisE, was performed using SP3-clinical proteomics, run on a ThermoFisher Orbitrap Fusion. For validation, tissue microarrays (n=52) were immunostained using three antibodies, ARID1A, Ubiquitin carboxyl-terminal esterase L1 (UCHL1) and Beta-catenin, selected based on availability and significant correlation to a specific EC subgroup. Ki-67 was used as a control stain. All cases and immunostains were scored by a senior pathology resident. Univariate analysis was performed comparing IHC expression between subgroups.

RESULTS: The average age of the cohort was 63.9 years (range: 33-87 years), all patients had a hysterectomy and the average size of the tumors was 4.3 cm (range: 1.4-12 cm). Full proteome analysis detected 2974 proteins with high confidence, 827 of them showed statistically significant enrichment for a molecular subgroup. ARID1A, UCHL1 and Ki-67 IHC showed good correlation with the mass spectrometry based analysis (p=0.047, 0.0011, 0.0032) whereas beta-catenin did not (p=0.44). Using IHC, UCHL1 differentiated p53 wt from the other subgroups (p=0.012).

CONCLUSIONS: Our preliminary data show that proteomic analysis using mass spectrometry can be translated successfully to IHC analysis to promote biomarker discovery. Tissue sampling, antibody quality and staining optimization likely account for some of the differences observed between the two assays. UCHL1 showed a significant correlation with the p53 wt group and might be a useful as part of an IHC panel for future subclassification. Further studies will validate the usefulness of UCHL1 in EC subtyping in a larger cohort as well as its potential prognostic significance.

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MICROGLIAL ENRICHMENT OF EMBRYONIC RODENT PRIMARY CORTICAL CULTURES USING GRANULOCYTE MACROPHAGE-COLONY STIMULATING FACTOR TO MODEL CENTRAL NERVOUS SYSTEM MICROGLIA

David Jure Hunt[1], Pierre Becquart[1], Jacqueline Quandt[1]

BACKGROUND/OBJECTIVES: Microglia are resident immune cells of the central nervous system responsible for several functions ranging from surveillance to synaptic pruning. Many neurodegenerative disorders, including multiple sclerosis, demonstrate pathological hallmarks which include chronic microglial activation. Establishing methods to culture microglia in vitro which represent their in vivo counterparts is essential to better understand roles for these cells in pathological conditions. A major limitation of traditional microglial protocols is small and contaminating populations. Mitogens such as granulocyte macrophage-colony stimulating factor (GM-CSF) have been described to induce microglial proliferation, however no published work has been reported characterizing the use of GM-CSF to establish microglia from embryonic rodent cortices. Here, we describe optimization of a protocol to grow microglia from embryonic day 18 (E18) rat and mouse primary cortical mixed glial cultures.

METHODS: Primary mixed glial cultures were prepared from E18 rat or mouse cortices plated at 100,000 cells/cm2. Cultures received a full media exchange at 1 day in vitro (DIV), 9 or 10 DIV, and a 50% top up weekly thereafter. 50 ng/mL of murine GM-CSF was added at 9, 10, 16, or 20 DIV, and cultures were subsequently harvested on 22, 24, or 30 DIV. Pipetting or shaking was used to harvest loosely adherent microglia followed by enumeration and assessment of viability to compare treatment regimens for cell yield with phase contrast microscopy to monitor cell morphology.

RESULTS: GM-CSF stimulation of rat mixed glial cultures on day 9 or 10 produced a 6.5-fold and 8-fold increase in microglial counts at 24 DIV compared to controls receiving no mitogen, respectively. Murine mixed glial cultures that received GM-CSF at 10 DIV and harvested at 24 DIV yielded more cells, nearly a 10-fold increase in microglia compared to untreated cultures. The addition of GM-CSF later in the culture period at 16 DIV with harvest at 22 DIV did not enrich microglial numbers to the same extent, but did increase them significantly. After 24 DIV microglia tended to become more adherent and more difficult to recover, such that attempts to extend culture as far as 30 DIV reduced, rather than enhanced yields.

CONCLUSIONS: We have established a promising protocol to enrich for high purity microglial populations from E18 murine and rat primary cortical mixed glial cultures using GM-CSF. Adding mitogens earlier in the culture period significantly enhances microglial recovery, but recovery is not increased by extending the overall time in culture; over time cells become adherent and more difficult to recover from the mixed glial adherent layer. Our work has optimized cell recovery and enrichment to yield significant numbers for in vitro studies, yet GM-CSF treatment of microglia has been observed by others to change morphology, enhance phagocytic activity and alter cytokine secretion of these cells. Work is ongoing to further characterize our GM-CSF-enriched microglial cultures to understand how representative they are of microglia in vivo both in healthy and pathological states.

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DETECTION OF NOVEL MARKERS OF TRANSITIONAL CELL CARCINOMA OF THE OVARY, THE TRANSITIONAL CELL CARCINOMA-LIKE VARIANT OF HIGH GRADE SEROUS CARCINOMA, USING PROTEOMICS AND IMMUNOHISTOCHEMISTRY

Jamie Magrill[1], [2]Basile Tessier-Cloutier, Stefan Kommoss[3], Blake Gilks[2], David Huntsman[1], Dawn Cochrane[1], Aline Talhouk[1], Robert Soslow[4], Gregg Morin[1], Chris Hughes[2], Anthony Karnezis[1], Christine Chow[2], Angela Cheng[2], Andreas du Bois[5], Jacobus Pfisterer[6] and Friedrich Kommoss[2]

BACKGROUND/OBJECTIVES: The current WHO classification does not separate transitional carcinoma of the ovary (TCCO) from conventional high grade serous carcinoma of the ovary (HGSC). TCCO has a better prognosis, possibly due to better chemosensitivity or less infiltrative growth pattern. The available immunohistochemical (IHC) markers do not differentiate between the two subtypes. Therefore, we sought to compare the proteomic profiles of conventional HGSC and TCCO to identify surrogate biomarkers of good prognosis from TCCO that could identify conventional HGSC tumors with a better prognosis.

METHODS: Full proteome analysis of 12 cases of TCCO and 12 cases of HGSC was performed using SP3-clinical proteomics, run on an ThermoFisher Orbitrap Fusion. For validation, tissue microarrays of TCCO (n=89) and HGSC (n=237) were immunostained with antibodies against proteins found to be enriched in TCCO. All cases and immunostains were scored by a gynecologic pathologist. Univariate analysis was performed comparing IHC expression in TCCO vs. HGSC.

RESULTS: We identified 1220 proteins that were significantly enriched in TCCO over HGSC. Claudin 4 and Ubiquitin carboxyl-terminal esterase L1 (UCHL1) were selected as potential markers of TCCO-like biology (p=0.0017, 0.0322). By IHC, Claudin 4 (95% confidence interval (CI) 0.171, 0.430) and UCHL1 (95% CI 0.291, 0.550) showed a significantly higher expression in TCCO as compared to HGSC (see Table 1).

Table·1¤	%·of·tumors·with·high·immunoreactivity·scores¤	
¤	Claudin·4¤	UCHL1¤
Pure·TCCO¤	34/59·(58%)¤	26/59·(44%)¤
Mixed·TCCO-HGSC,·TCCO·component¤	14/29·(48%)¤	8/29·(28%)¤
Mixed·TCCO-HGSC,·HGSC·component¤	19/28·(68%)¤	6/28·(21%)¤
Conventional·HGSC¤	33/235·(14%)¤	32/237·(14%)¤

Legend: Mixed TCCO: TCCO with minor component of conventional HGSC

CONCLUSIONS: Proteomic analysis showed differing protein profiles for TCCO and HGSC. By IHC, Claudin 4 and UCHL1 were identified as potential markers for TCC-like differentiation of high-grade serous carcinomas. Further studies will focus on the prognostic significance of these and other markers in larger cohorts of HGSC. This study presents a novel approach at identifying potential diagnostic, prognostic biomarkers and therapeutic targets. differential diagnoses, particularly desmoplastic small round cell tumor, as well as other gynecologic SRBCTs.

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HIGH-SENSITIVITY C-REACTIVE PROTEIN ASSAY UTILIZATION AT FRASER HEALTH HOSPITALS

Aria Shokoohi[1], Richard Cleve[1,2], Ramesh Saeedi[1,2]

BACKGROUND/OBJECTIVES: High-sensitivity C-reactive protein (hs-CRP) is more sensitive than a traditional C-reactive protein test. It is an inflammatory biomarker potentially linked to underlying atherosclerosis and cardiovascular disease (CVD). hs-CRP assays are used to assess CVD risk as they are able to quantitate CRP within the range normally seen in asymptomatic patients (<3 mg/L). The American Heart Association (AHA) does not recommended the test for general screening for heart disease, only those with an intermediate risk (a 10 to 20% chance of having CVD within the next 10 years). Patients with persistent values over 10 mg/L should be evaluated for non-cardiovascular sources of infection or inflammation. Due to the variability of values in an individual over time, the AHA guidelines suggest that the average of two hs-CRP assays optimally obtained two weeks apart would provide a more stable estimate of CVD risk than a single measurement.

METHODS: In this study, the utilization of hs-CRP assays was investigated at Fraser Health hospitals. Data analysis was carried out using Excel on 180,390 hs-CRP assays, which were ordered at 12 Fraser Health hospitals in 2016. The study primarily focused on the frequency of hs-CRP assay ordering for patients in the low, intermediate, and high-risk values, which were defined as <1, 1 to 3, and 3 to 10 mg/L respectively.

RESULTS: hs-CRP assays were ordered for 80,822 patients at 12 Fraser Health hospitals in 2016. From a total of 180,390 hs-CRP assays ordered, Surrey Memorial Hospital had ordered 92,071 (51.0%) hs-CRP assays, which was more than the other 11 Fraser Health hospitals combined. In terms of location, 82,842 (45.9%) of these hs-CRP assays were ordered from the emergency department and 76,596 (42.5%) were ordered for inpatients, with the remaining 11.6% being ordered for outpatients. Furthermore, 100,483 (55.7%) of these hs-CRP assays were found to be above 10 mg/L. According to the AHA guidelines, 19.7% of the total hs-CRP assays were at high risk, 12.9% at intermediate risk, and 11.5% at low risk. From a total of 2725 physicians, 1376 general practitioners and 134 emergency physicians ordered 80,677 (44.7%) and 52,622 (29.2%) hs-CRP assays respectively. As for the frequency of orders, 73,877 hs-CRP assays were repeat measurements (between 0-13 days) for the same patients prior to the optimal 2 weeks apart, suggested by the AHA.

CONCLUSIONS: hs-CRP assays are used to assess CVD risk, however most of these orders resulted in values over 10 mg/L which should prompt consideration of a source of infection or inflammation instead. The suggested two weeks optimal time period between repeated measurements by the AHA is not being complied with the physicians at Fraser Health hospitals.

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OXIDATIVE STRESS AND INFLAMMATORY MEDIATORS INFLUENCE EXPRESSION OF THE NEUROPROTECTIVE PROTEIN ARNT2 IN ASTROCYTES

Heather Yong[1], Pierre Becquart[2], Jacqueline Quandt[3]

BACKGROUND/OBJECTIVES: Multiple Sclerosis (MS) is a chronic inflammatory and neurodegenerative disorder of the central nervous system. The aryl hydrocarbon receptor nuclear transporter-2 (ARNT2) is a transcription factor with pleotropic effects influencing neuronal and axonal survival, including response to trauma, oxidative stress, or infection. We have previously shown that ARNT2 expression is altered in primary enriched neuronal cultures and in an experimental model of MS. We hypothesized that astrocytes also alter ARNT2 expression in response to environmental or inflammatory stressors, and examined ARNT2 regulation following serum deprivation or exposure to exogenous stressors.

METHODS: Primary enriched astrocyte cultures were established from embryonic day 18 cortices and were cultured for 14-16 days. They were first subjected to various horse serum (HS) concentrations in feeding medium to optimize the culture of astrocytes and response to serum exposure or deprivation at confluence. Exogenous stressors including hydrogen peroxide (H2O2, to model oxidative stress), ATP (to model cell signals), glutamate, and lipopolysaccharide (LPS, an inflammatory stimulus) were then applied and ARNT2 expression and cell morphology assessed by immunocytochemistry. Cell viability was tested by lactic dehydrogenase (LDH) release.

RESULTS: Primary enriched cultures of cortical astrocytes are 75-95% confluent and express negligible to low levels of ARNT2 when untreated. Reducing the HS concentration from the standard 10% composition to 0, 2, or 5 % over 24 hours caused significant changes in morphology, cell viability, and ARNT2 expression. Complete removal of serum increased the intensity of ARNT2 expression by 30% ($p=0.0024$), and corresponded with lower cytotoxicity measured by LDH release and altered morphology. H2O2 (25, 50 and 100 μM) exposure significantly increased ARNT2 protein levels: by 1h there was a 20% increase in ARNT2 in low H2O2 (25 or 50uM) treatment groups ($p<0.05$) that was sustained at 2h. In contrast, exposure to higher doses of H2O2 (100 μM) led to cell death with 23% cytotoxicity at 1 h and 53% at 4h. Conversely, LPS tended to lower ARNT2 expression without associated cell death, with the most marked reduction occurring at the highest concentration of LPS (1μg/mL). ATP and glutamate had negligible effects on ARNT2 expression in astrocytes.

CONCLUSIONS: To our knowledge, this is the first study of ARNT2 expression in glial cell populations, specifically astrocytes, either in vitro or ***in vivo***. Culturing astrocytes in serum-deprived media may be most representative of astrocytes in vivo, and increased ARNT2 associated with enhanced viability suggests it positive role in glial health. Regulation of ARNT2 in response to oxidative stress and acute inflammatory insult suggests a novel role in sensing stressors that may direct subsequent defense responses in the nervous system. Future studies to identify partners and downstream targets of ARNT2 regulation will further characterize its putative role in mediating glial as well as neuronal health during inflammatory and neurodegenerative processes in disorders such as MS.

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USAMA ABBASI

- *GRADUATE STUDENT*
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DESIGN OF A NOVEL LIVER TARGETED IRON CHELATING SYSTEM FOR THE TREATMENT OF TRANSFUSIONAL IRON OVERLOAD

Usama Abbasi[1], Srinivas Abbina[1], Jayachandran Kizhakkedathu[1, 2]

BACKGROUND/OBJECTIVES: Iron is essential for life. However, excess iron has an enormous damaging potential; it catalyzes the generation of reactive oxygen species, which leads to organ dysfunction. The ability to offload excess iron remains challenging since there is no known physiological excretion pathway. Transfusion therapy is the current standard of care for patients with myelodysplastic syndromes, sickle cell disease and thalassemia. Since humans cannot excrete iron, chronically transfused patients develop iron overload, which overwhelms the liver, a primary storage site, leading to organ toxicity and death. Iron chelation therapy uses low molecular weight drugs, however the inherent limitation is best exemplified by the current gold standard, desferrioxamine (DFO); short circulation, toxic, and non-specific tissue distribution. Novel treatment methods are needed to address this unmet clinical need. Thus, we hypothesize that a targeted macromolecular iron chelating system conjugated with liver targeting groups that achieves optimized blood circulation time would enhance the iron sequestration and excretion from the liver, and will eliminate iron-induced toxicity.

METHODS: The asialoglycoprotein receptor is predominantly expressed on hepatocytes and has a high affinity towards carbohydrate ligands. Building on our proof-of-concept long acting multifunctional polymeric iron chelating system, which efficiently excreted labile plasma iron, we will develop a novel macromolecular liver targeted iron chelator (LMLTCs). This system was optimized for ideal multivalent receptor interaction by controlling the number of carbohydrate residues on a polymeric scaffold. Three LMLTCs (LMLTC1, LMLTC2 and LMLTC3) were fluorescently labeled for uptake studies in HepG2 cells, a human hepatic cell line. Cells were incubated with varying concentrations of LMLTCs to determine EC50 values. Time profiles for the respective EC50 values were measured over 24 hours. Competitive uptake studies were performed with galactosamine to determine IC50 values. Additionally, non-specific binding was measured at 4ºC, and in a non-hepatic cell line (HeLa cells). LMNLTC, polymer conjugated to a non-specific ligand, served as a negative control. Flow cytometry was used for uptake studies and FlowJo was used to analyze data. Statistical analysis was performed on Graphpad Prism.

RESULTS: Hepatocytes internalized LMLTCs via receptor-mediated endocytosis; minimal uptake was observed in HeLa cells and at 4ºC. LMLTC2 and 3 had significantly lower EC50 values than LMLTC1. LMLTC2 demonstrated significant uptake compared to LMLTC3 over 24 hours. Based on LMLTC2's EC50 and IC50 values, the effective binding affinity was 290 NM.

CONCLUSIONS: To date, no methods are available to target iron chelators to susceptible organs. LMLTCs provide novel insight for the development of organ specific systems and have potential application for iron overload diseases. This system will be further optimized for chelating efficiency. Future experiments will address mechanisms of uptake and iron sequestration.

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ABHINAV AJAYKUMAR

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- *POSTER SESSION*
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HIV-EXPOSED UNINFECTED (HEU) NEWBORNS EXPOSED IN UTERO TO RITONAVIR-BOOSTED PROTEASE INHIBITOR ANTIRETROVIRAL THERAPY (ART) HAVE LOWER MTDNA CONTENT COMPARED TO ART-UNEXPOSED HEUS

Abhinav Ajaykumar[1,2], Izabelle Gadawska[1], Fatima Kakkar[3], Isabelle Boucoiran[4], Hugo Soudeyns[3,5], Ariane Alimenti[6], John Forbes[6], Hélène Côté[1,2]

BACKGROUND/OBJECTIVES: More than 37M people live with HIV globally, of which 48% are women who give birth to approximately 3.3M children every year. Combination antiretroviral therapy (cART) has been highly effective in controlling the virus among infected individuals. In the context of pregnancy, treatment with zidovudine (AZT) monotherapy during the mid-1990's, followed by the introduction of dual/triple ART has drastically reduced mother-to-child HIV transmission to ~1%. However, many ART drugs can cross the placenta and could affect cellular processes in the developing fetus. Some antiretrovirals such as Nucleoside Reverse Transcriptase Inhibitors (NRTIs) can inhibit the mitochondrial polymerase-γ enzyme that replicates mtDNA, while others such as Protease Inhibitors (PIs) can induce oxidative stress and/or affect mitophagy. These may result in mitochondrial alterations/dysfunction which can be reflected by compensatory changes in mtDNA content. Our objective was to investigate the impact of in utero ART exposure on infant blood mtDNA content at birth in two cohorts of HIV-exposed uninfected (HEU) and HIV-unexposed uninfected (HUU) infants.

METHODS: mtDNA content was measured via multiplex-qPCR in dried blood spots collected 0-5 days after birth from 104 HEU infants enrolled in the CMIS Mother-Child Cohort, and 68 HEU and 17 HUU infants in the CARMA Cohort. Univariate associations were investigated between mtDNA content and: infant sex, birth weight, gestational age (GA) at birth, ethnicity, preterm delivery (<37w gestation), maternal age at delivery, Hepatitis B/C co-infection status, smoking and illicit drug use during pregnancy, viral load closest to delivery, duration of ART during pregnancy and number of ART drugs during pregnancy (mono- vs. dual- vs. triple vs. ART naïve). Factors important univariately ($p<0.1$) were included in multivariable analyses.

RESULTS: Among the 172 HEU infants, 46 were exposed to maternal AZT monotherapy in utero, 21 to dual therapy (AZT+3TC (lamivudine)), 78 to triple therapy (n=66 2NRTIs+ritonavir-boosted PI, n=12 2NRTIs+ritonavir-sparing PI) and the remaining 27 were born to ART-naïve (untreated) mothers. In a multivariable analysis of all participants (n=189) that included GA, sex and maternal ART during pregnancy, higher mtDNA content was associated with lower GA ($p=0.002$) and being HEUs born to untreated ($p=0.006$), mono- ($p=0.047$), dual- ($p<0.001$) and ritonavir-sparing triple therapy ($p=0.026$) treated mothers (ref. HUU). In a similar model among HEUs only, infants exposed to ritonavir-boosted PI ART had 23% lower mtDNA content ($p=0.008$) compared to ART-unexposed HEUs.

CONCLUSIONS: Our results suggest that infant blood mtDNA content at birth is related to both maternal HIV and ART exposure during pregnancy. Increase in mtDNA among HEUs born to untreated, mono- and dual-NRTI treated mothers compared to HUUs may reflect compensatory mitochondrial proliferation in response to stresses. Ritonavir-boosted PI regimens appear to lower mtDNA content, possibly through failure to compensate, or increased oxidative stress and mitophagy leading to mitochondria elimination.

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HANI BAGHERI

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- *SUPERVISOR: DR. EVICA RAJCAN-SEPAROVIC*

IS THERE A MIRROR PHENOTYPE FOR 2P15P16.1 MICRODELETION SYNDROME?

Hani Bagheri[1,2], Ying Qiao[1,2], Xianghong Shan[3], Sally Martell[1,2], Suzanne Lewis[2,4], Cheryl Gregory-Evans[3], Evica Rajcan-Separovic[1,2]

BACKGROUND/OBJECTIVES: The 2p15p16.1 microdeletion syndrome has been described in 33 cases (Bagheri et al., 2016). Its core phenotype includes intellectual disability, microcephaly, hypotonia, delayed growth, common craniofacial features, and digital anomalies. Patient cell line and zebrafish studies identified three candidate genes XPO1, BCL11A, and REL. Their knock-down in zebrafish caused microcephaly, dysmorphic body, hindered growth, small fins and structural brain abnormalities. It was recently suggested that 2p15p16.1 microduplication causes mirror phenotypes and macrocephaly in carriers (Loviglio et al., 2016). Our aim was to test this possibility in a larger number of patients with the duplication and by overexpression of the 3 candidate genes in zebrafish.

METHODS: Clinical and genomic information for patients with 2p15p16.1 microduplication was extracted from DECIPHER. Overexpression of XPO1, REL and BCL11A in zebrafish embryos was performed by human RNA injection in 1-cell stage embryos. Head size, growth and body morphology was examined at 2 days post-fertilization.

RESULTS: Six patients were reported in DECIPHER with 2p15p16.1 duplications involving at least one of the 3 candidate genes and only 1 case had macrocephaly. Patients with microcephaly were also noted in this cohort ruling out a clear association of duplication and macrocephaly. Overexpression of XPO1 and REL in zebrafish did not cause phenotypic abnormalities, while BCL11A overexpression caused a hindered body growth and dysmorphic body trunk, but comparable head structure and size to controls.

CONCLUSIONS: Our studies do not support the existence of mirror phenotypes in cases with 2p15p16.1 duplication. The overexpression of BCL11A, however, may be associated with body dysmorphology but not head size anomaly.

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ASMA BASHIR

- *GRADUATE STUDENT*
- *POSTER SESSION*
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DEFINING THE BIOMECHANICAL AND BIOLOGICAL THRESHOLD OF MURINE MILD TRAUMATIC BRAIN INJURY USING CHIMERA (CLOSED HEAD IMPACT MODEL OF ENGINEERED ROTATIONAL ACCELERATION)

Dhananjay Namjoshi[1], Wai Hang Cheng[1], Asma Bashir[1], Anna Wilkinson[1], Sophie Stukas[1], Kris Martens[1], Tom Whyte[2,3], Zelalem Abebe[2,3], Kurt McInnes[2,3], Peter Cripton[2,3], Cheryl Wellington[1]

BACKGROUND/OBJECTIVES: CHIMERA (Closed Head Impact Model of Engineered Rotational Acceleration) is a recently described animal model of traumatic brain injury (TBI) that primarily produces diffuse axonal injury (DAI) characterized by white matter inflammation and axonal damage. CHIMERA was specifically designed to reliably generate a variety of TBI severities using precise and quantifiable biomechanical inputs in a nonsurgical user-friendly platform. The objective of this study was to define the lower limit of single impact mild TBI (mTBI) using CHIMERA by characterizing the dose-response relationship between biomechanical input and neurological, behavioral, neuropathological and biochemical outcomes.

METHODS: Wild-type male mice aged 4-5 months were subjected to a single CHIMERA TBI using six impact energies ranging from 0.1 to 0.7 J, and post-TBI neurological, behavioral, neuropathological and biochemical outcomes were assessed at 6h, 1d, 2d, 7d, and 14d time-points.

RESULTS: We report that single TBI using CHIMERA induces injury dose- and time-dependent changes in behavioral and neurological deficits, axonal damage, white matter tract microgliosis and astrogliosis. Impact energies of 0.4 J or below produced no significant phenotype (sub-threshold), 0.5 J led to significant changes for one or more phenotypes (threshold), and 0.6 and 0.7 J resulted in significant changes in all outcomes assessed (mTBI). We further show that linear head kinematics are the most robust predictors of duration of unconsciousness, severity of neurological deficits, white matter injury, and microgliosis following single TBI.

CONCLUSIONS: Our data extend the validation of CHIMERA as a biofidelic animal model of DAI and establish working parameters to guide future investigations of the mechanisms underlying axonal pathology and inflammation induced by mechanical trauma.

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EMILY BUTTON

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. CHERYL WELLINGTON*

HIGH-DENSITY LIPOPROTEINS SUPPRESS AMYLOID BETA-INDUCED ACTIVATION OF HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

Emily Button[1,2], Jerome Robert[1,2], Sophie Stukas[1,2], Guilaine Boyce[1,2], Ebrima Gibbs[2,3], Catherine Cowan[4], Wai Hang Cheng[1,2], Sonja Soo[1,2], Brian Yuen[1,2], Arvin Bahrabadi[1,2], Kevin Kang[1,2], Iva Kulic[1,2], Gordon Francis[5], Neil Cashman[2,3], Cheryl Wellington[1,2#]

BACKGROUND/OBJECTIVES: Epidemiological studies suggest a link between plasma high-density lipoprotein (HDL) cholesterol levels and Alzheimer's disease (AD) risk through mechanisms that are not understood. We hypothesize that HDL protects against AD through actions at the blood-brain-barrier. HDL has vasoprotective functions in large peripheral arteries, however, it is unknown if these functions extend to cerebral vessels to reduce the contribution of cerebrovascular dysfunction in AD pathogenesis. We investigated in vitro interactions between HDL and amyloid beta (Aβ), the toxic peptide known to accumulate in AD, in peripheral and brain-derived endothelial cells (EC).

METHODS: HDL was isolated by density gradient ultracentrifugation and added to human umbilical vein endothelial cells (HUVEC) or human cerebral microvascular endothelial cells (hCMEC/D3). Cell activation was measured by counting adhered labelled peripheral blood mononuclear cells (PBMC) after stimulation with tumour necrosis factor α (TNFα) or Aβ. Aβ binding and uptake into cells was measured using ELISA and immunofluorescence. All experiments included at least 4 independent replicates.

RESULTS: We demonstrate that HDL attenuates Ab-induced EC activation independent of nitric oxide production, miR-233 and changes in adhesion molecule expression. Rather, HDL acts through scavenger receptor BI to block Ab uptake into ECs and, in vitro, can maintain Ab in a soluble state. We validated our results using three dimensional engineered vessels composed of primary human endothelial and smooth muscle cells. Following Ab addition to the abluminal (brain) side, we demonstrated that HDL circulated within the lumen attenuates EC activation, again independent of intracellular adhesion molecule changes.

CONCLUSIONS: We show that the anti-inflammatory activities of HDL extend to cerebrovascular endothelial cells and work to suppress Aβ-induced activation through a novel mechanism involving the inhibition of Aβ binding and uptake into cells through SR-BI. The protective role for HDL against Aβ may explain the epidemiological evidence supporting a protective effect of high plasma HDL cholesterol levels against dementia.

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YUTING SHARY CHEN

- *GRADUATE STUDENT*
- *POSTER SESSION*
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ALISERTIB AS A THERAPEUTIC POTENTIAL FOR SMALL CELL CARCINOMA OF THE OVARY, HYPERCALCEMIC TYPE

Yuting Shary Chen[1], Yemin Wang[1], David Huntsman[1, 2]

BACKGROUND/OBJECTIVES: Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a rare and aggressive malignant neoplasm that is found in women mostly in their 20s. With such low survival rate (1-year: 50%; 5-year: 10%), therapeutic solution for SCCOHT is urgently needed. Several groups, including our lab, have found the genetic inactivation of SMARCA4 is presented in over 90% of the SCCOHT cases. Recently, some groups reported that SMARCA4-deficient tumors are heavily depend on aurora A kinase (AURK) for mitotic spindle assembly. Pharmacological inhibition of AURK by clinical applicable inhibitors have been shown to robustly suppress the tumor growth in vitro and in vivo in both non-small cell lung cancer and rhabdoid tumor with SMARCA4 deficiency.
We hypothesize that SMARCA4-deficient SCCOHT is sensitized to AURK inhibitor and use of orthotopic xenograft models of SCCOHT will be more valuable for testing the efficacy of anti-cancer drugs for SCCOHT than subcutaneous xenograft model.

METHODS: Fluorescent-labelled SCCOHT cell lines, including BIN-67, SCCOHT-1 and COV-434 will be seeded in 96-well plates for drug sensitivity assays. Cell viability will be monitored by crystal violet stain. Fluorescent-labelled cell lines will also be injected into mice subcutaneously and intrabursally into ovary respectively. Tumor growth will be measured biweekly with the bio-imaging system. Mice serum before the initial treatment and after the last treatment will also be collected for calcium level comparison to see if hypercalcimia is presented in the model. Tumors will be harvested at the end of study for histology comparison between the two models. Alisertib efficacy study will be conducted in the SCCOHT intra bursa model. Drug will be delivered daily for consecutive 6 days with a one-day break for 3 consecutive weeks.

RESULTS: SMARCA4-deficient SCCOHT cell lines are sensitized to AURK inhibitor, Alisertib, ***in vitro***, at a dose-dependent manner. Fluorescent SCCOHT cell lines have been successfully established, and demonstrated similar characteristics as the parental cell lines, in respects of growth rate, drug response and cell morphology. Intra bursa model is under optimization process, and further experiment involving comparison between different mouse model will be performed.

CONCLUSIONS: Our preliminary results shows that Alisertib sensitizes the SMARCA4-deficient SCCOHT cell lines in vitro. The development of intro bursa model for testing Alisertib efficacy will be crucial for predicting the potential clinical drug response. Combination therapy with Alisertib will also be considered for further studies. Ultimately, we hope this can lead to launching of therapeutic drugs into clinical trial to benefit the SCCOHT patients, who are desperate for the effective treatment.

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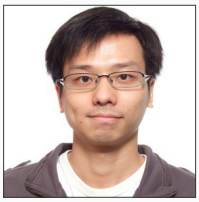

WAI HANG CHENG

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. CHERYL WELLINGTON*

AGE HAS A GREATER INFLUENCE THAN AMYLOID BURDEN ON THE ACUTE RESPONSE TO MILD TRAUMATIC BRAIN INJURY IN MICE

Wai Hang Cheng[1], Sophie Stukas[1], Kris Martens[1], Dhananjay Namjoshi[1], Emily Button[1], Anna Wilkinson[1], Asma Bashir[1], Jerome Robert[1], Peter Cripton[2], Cheryl Wellington[1]

BACKGROUND/OBJECTIVES: Incidence of traumatic brain injury (TBI) peaks in young and old individuals, and older age is associated with worse outcome and poorer recovery. TBI is also an established risk factor for neurodegenerative conditions such as dementia and chronic traumatic encephalopathy (CTE), and much remains to be learned about how age at injury affects susceptibility to neurodegeneration. We aimed to delineate how TBI, age at injury, and genetic predisposition to amyloid deposition interact.

METHODS: Repetitive mild TBI was induced in APP/PS1 and wildtype (WT) mice using the CHIMERA model at 6 or 13 months of age, and acute behavioural, histological and biochemical changes were assessed up to 14 days post-injury.

RESULTS: We observed impaired post-TBI spatial learning in old but not young mice regardless of genotype, and age-dependent post-TBI changes in Ab deposition in APP/PS1 mice. Age at injury and genotype showed a complex interaction with respect to cytokine and microglial responses, where the neuroinflammatory response is exacerbated in young APP/PS1 but blunted in old APP/PS1 mice, whereas in WT mice the response is sensitized by older age.

CONCLUSIONS: Both age at injury and APP/PS1 genotype modify TBI outcomes, with chronological age at injury being the more robust modifier.

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JENNA COLLIER

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. KEVIN BENNEWITH*

THE THERAPEUTIC DEPLETION OF EOSINOPHILS MAY DECREASE PULMONARY METASTASIS IN MOUSE MODELS OF BREAST CANCER

Jenna Collier[1,2] and Kevin Bennewith[1,2]

BACKGROUND/OBJECTIVES: It is estimated that over 90% of cancer-related deaths are associated with the development and growth of tumour metastases, which occurs when cancer cells dissociate from the primary tumour and establish secondary masses in distant tissues. Various resident and recruited immunosuppressive cells are critical in facilitating immune-escape and promoting the growth of these metastases. However, the role of eosinophils in pulmonary metastasis remains poorly defined by the existing literature. Eosinophils are multifunctional innate immune cells containing cytotoxic proteins and chemical mediators that are capable of exerting either pro- or anti-tumour effects. Much of the role of eosinophils in pulmonary metastasis has been defined using the IL-5 knockout mouse, which lack the cytokine IL-5 that is necessary for eosinophil development. However, low numbers of eosinophils are still produced in IL-5 knockout mice and IL-5 has pleiotropic effects on other cell types, complicating the use of these mice to study eosinophil function. We will use novel ddGATA/IL-5Tg mice that are deficient in eosinophils and immunological depletion of eosinophils in wild-type mice to analyze the influence of eosinophils on primary and metastatic mammary tumour growth. We hypothesize that eosinophils contribute to the development of pulmonary metastases in the lungs and therapeutic depletion of eosinophils using anti-IL-5 immunotherapy is an effective strategy to decrease metastasis in mouse models of breast cancer.

METHODS: Eosinophils were quantified in the lungs and tumours of mice bearing various metastatic mammary tumours by immunofluorescence or flow cytometry as CD11b+Siglec-F+ cells. Growth of orthotopic E0771-LMB mammary tumours in both wild-type and ddGATA/IL-5Tg mice were monitored by caliper measurements twice per week and surgical resections of the primary tumour were performed at 2 weeks post-implant to allow additional time for the metastatic lesions in the lung to develop. Pulmonary metastases in the lungs were enumerated by histology.

RESULTS: Eosinophils are increased in the lungs of mice bearing metastatic mammary tumours relative to naïve controls and are present within the primary tumour. Eosinophils do not have a significant effect on the growth of the primary tumour or the development of pulmonary micrometastases in ddGATA/IL-5Tg mice bearing E0771-LMB mammary tumours. We are currently investigating the efficacy of immunological depletion of eosinophils in alternative mouse models of metastatic breast cancer syngeneic to the Balb/C background are ongoing.

CONCLUSIONS: Although eosinophils do not appear to have a significant role on the development of pulmonary micrometastases, it has yet to be determined whether they may limit the growth of macrometastases. Our research intends to delineate the role of eosinophils in pulmonary metastasis evaluate the efficacy of anti-IL-5 immunotherapy on metastatic disease.

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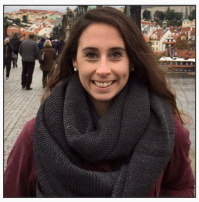

LISA DECOTRET

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. CATHERINE J. PALLEN*

PROTEIN TYROSINE PHOSPHATASE ALPHA (PTPA) PROMOTES INVADOPODIA-MEDIATED CANCER METASTASIS

Lisa Decotret[1,3] and Catherine Pallen[1,2,3]

BACKGROUND/OBJECTIVES: Normal cell migration is a highly conserved process that plays a critical role in many physiological processes such as wound healing. Aberrant cell migration is implicated in cancer metastasis, which is the leading cause (90%) of cancer-related mortality. In normal cells, cell migration is a dynamic process characterized by the cyclical adhesion to and release from the extracellular matrix (ECM), and involves cellular structures known as focal adhesions. In highly invasive tumour cells, the ability to invade is mediated by similar integrin-mediated structures called invadopodia. These are dynamic Src-regulated, actin-based protrusions of the plasma membrane that mediate ECM degradation. Protein tyrosine phosphatase alpha (PTPa), a widely expressed transmembrane protein, positively regulates integrin signaling and promotes cell migration. PTPa has been shown to be a critical Src activator and that subsequent Src-mediated phosphorylation of PTPa at Tyr789 is required for normal cell migration. However, little is known about the role of PTPa in cancer cell motility. Our goal is to determine the roles of PTPa in Src-signaling mechanisms that regulate invadopodia structure and function to promote the invasive motility of malignant cells.

METHODS: Small interfering-RNA (siRNA) was used to deplete PTPa in MDA-MB-231 (breast cancer) cells, and siRNA-resistant wildtype (WT)-PTPa and mutant-PTPa-Y789F (mutant) expressing plasmids were reintroduced into PTPa-null cells. These cells were then used in Transwell migration and invasion assays to investigate the role of PTPa/PTPa-Y789 phosphorylation in tumour cell motility. An invadopodia-mediated ECM-degradation assay was used to determine the degradation ability of control vs. PTPa-null cells. Lastly, MDA-MB-231 cells were transfected with GFP-PTPa and imaged using confocal microscopy to determine if PTPa localizes to the invadopodia-specific marker cortactin. Statistical analysis was performed using a Student's t-test.

RESULTS: PTPa-null MDA-MB-231 cells showed reduced migration ($p= 0.01$, $n=6$) and invasion ($p=0.008$, $n=3$) through the Transwell membrane compared to the control. This effect was rescued upon reintroduction of WT-PTPa; however it was not reversed by re-expressing the mutant. This suggests phosphorylation of PTPa at Tyr789 promotes both tumour cell migration and invasion. The invadopodia-mediated ECM-degradation assay revealed that PTPa-null cell (16.69%) have an impaired ability to degrade ECM compared to the control (51.51%), indicating PTPa promotes invadopodial function ($p=0.0004$, $n=4$). Furthermore, PTPa co-localizes with cortactin to punctate structures, suggesting PTPa is present in invadopodia..

CONCLUSIONS: These preliminary data have shown that PTPa is present within invadopodial structures and positively regulates invadopodia-mediated tumour cell motility. Future research may reveal new mechanistic targets for therapeutic intervention to prevent cancer metastasis and limit mortality.

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NATALIE FIRMINO

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HYPOXIA IS INDUCED IN TUMOR-DRAINING LYMPH NODES AND AFFECTS LOCAL LYMPHOCYTE POPULATIONS

Natalie Firmino[1,2] Kevin Bennewith[1,2]

BACKGROUND/OBJECTIVES: Lymph node metastasis has historically been considered a passive process, with cancer cells draining through the lymphatic system and becoming trapped in downstream lymph nodes; however, recent evidence suggests that the development of metastases in the tumour-draining lymph nodes (TDLN) involves pre-metastatic changes in the lymph node microenvironment. Such changes include the dilation of the subcapsular sinus (SCS), a region of the lymph node that lymph-borne tumour cells first encounter. Since the SCS is devoid of blood vessels, we hypothesized that tissue near the SCS of TDLNs and therefore local immune cell populations are hypoxic, resulting in impaired anti-tumour immune responses and a permissive environment for metastatic colonization of the lymph node.

METHODS: Murine mammary carcinoma cells (4TO7) were injected into the mammary fat pad of immune-competent BALB/c mice. After the establishment of mammary tumours (3 weeks post-tumor implant), the hypoxia-specific marker pimonidazole was administered to label all hypoxic cells in tumour-bearing and control mice. After harvesting the inguinal and axillary lymph nodes draining the mammary fat pad, bound pimonidazole was measured using flow cytometry or immunofluorescence microscopy. Flow cytometry was also used to assess whether immune cell populations became hypoxic in either control or tumor-draining lymph nodes.

RESULTS: Immunofluorescence microscopy revealed that both tumor-draining inguinal and axillary lymph nodes contained hypoxic tissue, which was primarily located in the lymph node cortex. Lymph node hypoxia was induced by the tumor, since hypoxia was absent in lymph nodes of non-tumor bearing mice. Flow cytometry analysis of whole lymph node cell suspensions confirmed these findings, showing that the percentage of hypoxic cells was significantly greater in tumor-draining lymph nodes relative to controls, and that hypoxia developed in approximately 60% of tumor-draining lymph nodes. Lymph node hypoxia exposed a subset of local B (CD19+) and T (CD3+) lymphocytes to low oxygen levels, which may subsequently affect these cells' phenotype and local anti-tumor immune responses. Finally, the extent of hypoxia in tumor-draining axillary lymph nodes was positively correlated with primary tumor weight ($r^2=0.3956$, $p=0.0013$).

CONCLUSIONS: Lymph nodes draining murine mammary tumors develop hypoxic regions that affect local B and T lymphocyte populations. Current work is assessing whether exposure to tumor antigens is sufficient to induce lymph node hypoxia, and how the oxygen-poor environment impacts anti-tumor immune responses. Our findings add to the repertoire of tumour-induced changes in the lymph node microenvironment, and may have important implications for the efficacy of anti-cancer immunotherapy.

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SLOWING THE PREMATURE AGING PROCESS: THE BENEFITS OF VIRAL CONTROL AND SMOKING CESSATION ON TELOMERE LENGTH

Anthony Hsieh[1,2], Clara Van Ommen[3], Elana Kimmel, Melanie Murray[4,5,6], Neora Pick[4,5,6], Ariane Alimenti[7,8], Jason Brophy[9], Fatima Kakkar[10], Ari Bitnun[11], Hélène Côté[1,2,5,7], and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

BACKGROUND/OBJECTIVES: Telomeres are nucleoprotein complexes at the ends of chromosomes that are truncated with each cell division. Consequently, telomeres also shorten over lifespan, and shorter leukocyte telomere length (LTL) is a marker of cellular aging associated with age-related physical morbidities, cognitive aging, and mortality. Infection with Human Immunodeficiency Virus (HIV) and cigarette smoking are both associated with LTL cross-sectionally, but their influence on LTL over time is unclear. This study investigated the relative contribution of HIV infection, the amount of HIV in blood (HIV viral load), and smoking on LTL dynamics in people living with HIV.

METHODS: All non-pregnant girls and women ≥12 years old enrolled in the Children and Women: AntiRetrovirals and Markers of Aging (CARMA) cohort with available blood specimens were included. For those with >1 sampling ≥1 year apart, the latest specimens were included in longitudinal sub-analyses. LTL was measured by monochromatic multiplex qPCR. Possible predictors including age, ethnicity, smoking (current, past, never), HIV/viral load (VL) status (HIV-, HIV+/detectable VL, HIV+/undetectable VL), peak VL, and Hepatitis C virus status were considered for inclusion in multivariable models.

RESULTS: LTL was obtained for 287 HIV+ and 211 HIV- participants aged 12-78 years, including 199 HIV+ and 49 HIV- with two specimens 1.0-7.9 years apart. In a cross-sectional multivariable regression, shorter LTL was associated with older age (beta=-0.35, p<0.0001), current smoking (beta=-0.18, p=0.001) vs. never, and HIV+/detectable VL (beta=-0.13, p=0.004), but not HIV+/undetectable VL (beta=-0.06, p=0.17) vs. HIV-, after adjusting for ethnicity (n=450, R^2=0.25). These results persisted in sensitivity analyses that either excluded ethnicity, restricted ethnicity to the largest group, or restricted age to ≥16 for consideration of smoking status. Longitudinally, LTL attrition rates were greater with current smoking (beta=-0.20, p=0.004) vs. never, but not associated with baseline HIV/VL status, after adjusting for baseline LTL (n=246, R^2=0.11). HIV+ participants with detectable VL who became undetectable at follow-up were more likely to show an increase in LTL and vice-versa (n=57, Fisher's exact test, p=0.043).

CONCLUSIONS: These results highlight the negative impact of current smoking on LTL, with an effect size larger than even uncontrolled HIV infection. These data suggest that LTL is better preserved in controlled HIV, and stress the importance of smoking cessation and controlling viremia to curb cellular aging.

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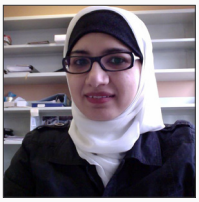

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EXPLORING DYNAMICS OF GENOMIC CLONES FOR BIOMARKERS OF SENSITIVITY AND RESISTANCE IN BREAST CANCER PRE CLINICAL MODELS

Farhia Kabeer[1,2], Hong Xu[1,2], Peter Eirew[2], Damian Yap[1,2], Derek Chiu[2], Steven McKinney[2], Sam Aparicio[1,2]

BACKGROUND/OBJECTIVES: Breast cancer is an ecosystem of genetically diverse evolving clones, which emerge in time and space. Genomic instability results in diversity, which promotes clonal dynamics (the expansion and decline of clonal populations)and and clonal evolution (appearance of new clones). Subdominant mutations in known breast tumor suppressors and oncogenes gives critical motivation to ascertain a means for determining which clones in any specific molecular subtype will be biologically relevant to the disease, such as those with the genotypes that confer risk of progression or drug resistance. We are interested in understanding clonal evolution and to develop experimental and mathematical framework for predicting the likely trajectories of clones in patients after drug treatment. To explore clonal dynamics we are interested in two drugs: First, piloting the approach using standard drug agents such as Paclitaxel, given as first line of treatment in most of cancers and secondly, a novel compound-CX5461. We have recently demonstrated that CX5461 is a G-quadruplex binder and synthetically lethal with defective homologous recombination DNA repair pathway. We want to explore the relationship between DNA repair pathways and clonal evolution and specifically how it diversifies with drugs. Our over-arching hypotheses are the cells with defective DNA repair pathways are more sensitive to G4 stabilizers and by exploring clonal dynamics of patient derived xenografts (PDX), we can predict biomarkers of sensitivity and resistance to drugs.

METHODS: For *in vitro* sensitivity mechanism of CX5461 and other conventional chemotherapies, HCT116 DNA-pk-/-, HCT116 LIG4-/- and PDX dissociated tumor cells were used in short term culture assay. WST-I reagent and clonogenic assay were used to measure cellular proliferation and viability. For *in vivo* we are developing methods of measuring clonal trajectories in patient-derived xenografts (PDX), as a first step to developing predictive models. A big cohort of same molecular subtype of PDX was made and treated with CX5461 and tumors were harvested at different time points to track the clones. We are trying to map the relationships between clones and drug sensitivity by combining deep sequencing with whole genome/single cell sequencing analysis and mathematical approaches to quantifying dynamics and fitness.

RESULTS: Our initial results show that [1] BRCA1/2-/-, DNA-pk-/- and LIG4-/-HCT116 cells are more sensitive to CX5461 compared to wild type HCT116 cells. These results of ours lead to the conclusion that BRCA and NHEJ pathways are required for the repair of CX5461 induced DNA damage and failure to do so leads to lethality [2] More importantly, using this novel agent along with standard agents we have some preliminary ideas on how we will be able to observe clonal responses to drugs in polyclonal PDX that can mirror original patient's tumor dynamics.

CONCLUSIONS: Our study will be able to quantify the critical measure of signs and properties of cells or group of cells that will be exhibiting survival advantage or disadvantage in response to Paclitaxel and the new compound CX-5461.

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MALT1 IS CRITICAL FOR DIFFERENTIATION OF IFN-G AND IL17- SECRETING T CELLS

Sohyeong Kang[1,2], Yu-Husan Huang[1,2], Kevin Tsai[1,2], Shan-Yu Fung[1], Stuart Turvey[1], John Priatel[1,2]

BACKGROUND/OBJECTIVES: Primary immunodeficiency diseases (PIDs) are genetic disorders in which components of the human immune system being absent or dysfunctional resulting in increased susceptibility to infection, autoimmunity or malignancy. Recently, a child admitted to British Columbia Children's Hospital presented an immune dysregulation, displaying recurrent infections with severe inflammation of the gastrointestinal tract. Whole-exome sequencing revealed that the patient inherited a missense mutation in MALT1 (mucosa-assosicated lymphoid tissue lymphoma-translocation gene 1) and her lymphocytes exhibited deficits in MALT1 protein and MALT1- dependent signal transduction. MALT1 plays critical roles in NF-kB pathway upon T cell receptor signal transduction in at least two different mechanisms: (1) as a signaling adaptor molecule bringing CARMA-1 and BCL-10 to form the CBM signalosome complex and (2) as a caspase-like protease (paracaspase) cleaving substrates after arginine residues. Here, we investigate that the role of MALT1 in T cell signal transduction and function.

METHODS: Cryogenically frozen CD4 and CD8 T cells from the patient ($MALT1^{mut/mut}$), patient's unaffected sibling ($MALT1^{mut/WT}$) and age/sex-matched control ($MALT1^{WTWT}$) were expanded with irradiated feeder cells for further experiments. At Day 7 post-stimulation, cells were rested overnight and PMA (100ng/ml)/Ionomycin (1ul/ml) or CD3/CD28 activator bead stimulation was given to examine cytokine production and T cell phenotype.

RESULTS: We have found that the patient CD4 T cells exhibit defects in differentiating into IFN-g and IL-17 secreting effector T cells and these impairments could be responsible for inefficient immune responses leading to chronic infections.

CONCLUSIONS: Our findings suggest how MALT1-deficiency may result in inefficient immune responses and chronic infections. A better understanding of MALT1 will provide insight into inflammatory disease and prove invaluable in supporting MALT-targeted therapies to modulate T cells to treat autoimmune diseases.

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GENOMIC SCREEN OF CISPLATIN RESISTANCE GENES IN MUSCLE-INVASIVE BLADDER CANCER USING GENOME-SCALE CRISPR KNOCKOUT

Gunjan Kumar[1,3], Beibei Zhai[1,3], Peter Black[1,2,3], Mads Daugaard[1,2,3]

BACKGROUND/OBJECTIVES: Bladder cancer is the 4th most common cancer in men and 9th most common in women with incidences on the rise. The overall five-year survival averages 50% for non-metastatic and 6% for metastatic disease. Neoadjvuant chemotherapy (NAC) followed by radical cystectomy has been shown in multiple trials and meta-analyses to improve five year survival, and is therefore part of standard therapy in patients. However, 60% of patients are inherently resistant to NAC tumour in the bladder at the time of cystectomy. While several mechanisms of resistance have been proposed, the causal genetic modifications remain largely unknown. The objective of this study is to take a functional genomic approach to identify genetic components that confer treatment resistance in bladder cancer. Our central hypothesis is that using a genome-wide CRISPR knockout library, we can identify specific gene targets that confer cisplatin resistance to bladder cancer cells.

MATERIALS & METHODS: To test this hypothesis, we take advantage of a genome-wide CRISPR knock-out (GeCKO) pooled library targeting 18,080 genes with 64,751 synthetic guide RNA (sgRNA). Lentivirus are made by co-transfecting the lentiCRISPR plasmid (plasmids expressing the sgRNA) with packaging and envelop plasmids into HEK293T cells. We then transduce UC13 cells (derived from a lymphatic metastasis bladder transitional cell carcinoma) with the lentivirus at a multiplicity of infection (MOI) of 0.4 (number of virions per cell). Successfully transduced cells are selected using puromycin and allowed to repopulate before being subjected to a cisplatin selection pressure. Cells that survive cisplatin selection are then harvested, and their genomic DNA is extracted using a gDNA extraction kit. We will then perform PCR to amplify the sgRNAs and to attach barcodes in preparation for an Illumina deep sequencing which will identify knocked-out genes.

RESULTS: We were able to produce lentivirus particles at a concentration of 2 million units/mL. Upon transduction, selection and subsequent dose response assay to cisplatin, we were able to show that there was a significant increase in IC50 values of the UC13 cells between the transduced and non-transduced cells (3.0 uM vs. 1.3 uM) indicating a successful increase in cisplatin resistance.

CONCLUSIONS: These results provide validation that the GeCKO is a powerful tool that will allow us to elucidate genes that are major players in tumours that are resistant to cisplatin treatment in bladder cancer with an increased accuracy. This will give us invaluable insights into the genetic mechanisms underlying a resistant phenotype, and in the future, allow for the development of novel interventions to improve outcomes for patients with chemo-resistant bladder cancer.

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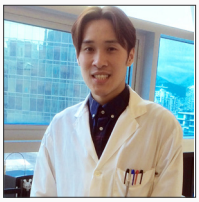

JACKY LEUNG

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TARGETING PROLINE ISOMERASE PIN1 FOR THE TREATMENT OF CASTRATION-RESISTANT PROSTATE CANCER

Jacky Leung and Marianne Sadar

BACKGROUND/OBJECTIVES: The androgen receptor (AR) remains an important therapeutic target throughout all stages of prostate cancer. As prostate cancer advances to lethal, castration-resistant prostate cancer (CRPC) there is the reappearance of transcriptionally active AR. This persists despite continued androgen deprivation therapy, aimed at maintaining castrate levels of serum androgen. This resurgence of active AR is presumably driven by the AR N-terminal domain (NTD), which harbors a powerful transactivation domain that can activate transcription in the absence of androgen. An intriguing property of the AR NTD is that its structure is intrinsically disordered, however it carries putative binding sites for Pin1, a proline isomerase that changes protein structures. Since the innate ability of the AR NTD to adopt transient structures is important for activation, we hypothesized that Pin1 regulates motifs located on the AR NTD.

METHODS: Several known inhibitors of Pin1 were used to study the function of Pin1 in androgen-sensitive (LNCaP) and androgen-independent (LNCaP95) prostate cancer cells. Luciferase reporter gene assays were used to measure the transcriptional activity of AR induced by androgen, or transactivation of the AR NTD. BrdU incorporation assays were employed to assess cell proliferation.

RESULTS: Our results demonstrated that inhibition of Pin1 interrupted the function of the AR NTD. Targeting Pin1 effectively blocked transcription mediated by AR induced by androgen, as well as transactivation of the AR NTD in the absence of androgen. Furthermore, inhibition of Pin1 attenuated the growth of prostate cancer cells driven by AR.

CONCLUSIONS: Here we describe that Pin1 is a critical factor for transcription mediated by AR, by acting on the AR NTD. Since there is no cure for CRPC, detailed understanding of the molecular mechanisms that contribute to AR signaling in the castrate environment will aid the development of more effective therapies for prostate cancer.

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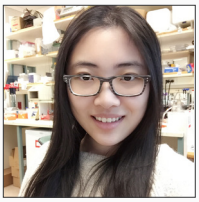

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FUNCTIONAL CHARACTERIZATION OF INTEGRIN-A6 ISOFORMS IN TUMORIGENESIS OF BREAST CANCERS

Ling Li[1,4], Karen Jung[2,4], Pascal Leclair[2,4], Heidi Vieira[3], Christopher Maxwell[2,4], Chinten James Lim[2,4]

BACKGROUND/OBJECTIVES: Integrins are a family of heterodimeric cell surface adhesion receptors each composed of an alpha- and a beta-subunit, both having a globular extracellular domain for interaction with ligands (including extracellular matrix), a single pass transmembrane domain, and a cytoplasmic tail for transduction of intracellular signaling. Alpha6 subunit (aka CD49f) dimerizes with either beta1 or beta4 subunits to generate alpha6beta1 or alpha6beta4 integrins, respectively, and specifically recognizes laminin. Expression of alpha6 integrins has commonly been used as a biomarker for embryonic and adult tissue-specific stem cells, as well as for cancer stem cells (CSCs) of breast and other cancers. Despite a central status as a CSC marker, the biological roles of alpha6 integrins remain largely unexplored. Recent work has identified two further subpopulations within CD44hi/CD24lo-enriched breast CSCs, with high alpha6A expression showing the epithelial phenotype and high alpha6B expression showing the more tumorigenic, mesenchymal phenotype. Alpha6A and 6B are splicing variants of the alpha6 mRNA, with the only difference resides in the exons possessed in their cytoplasmic tails, which are important in governing intracellular signaling. We hypothesize that alpha6 integrin contributes to the stem-like properties in breast cancer cells with its isoforms alpha6A and alpha6B exerting different effects due to distinct cytoplasmic tails mediating distinct intracellular signaling.

METHODS: We will use triple negative breast cancer cell lines MDA-MB-231 and SUM-149 that have endogenous expression (alpha6 WT), lack expression (alpha6 null), or have isoform-specific expression (alpha6A or 6B). CRISPR/Cas9 gene editing system is used to knock-out expression of both isoforms by targeting one of the early extracellular exon/s. Alpha6 null is used to re-express specific alpha6 isoforms at levels comparable to parental alpha6 WT (sorted by FACS). The stem cell population is characterized by CD44hi/CD24lo using FACS. Adhesion-mediated intracellular signaling pathways are induced by adhesion to laminin, with downstream proteins in stem cell relevant pathways (Wnt, TGFb) assessed by western blot assays. Basal or luminal phenotypes are determined by biomarkers (CK5, EGFR). Two-dimensional cell proliferation and drug resistance is assessed by MTS assay. Tumorigenic potential is assessed by tumorsphere formation assay. Cell invasion is assessed by transwell migration and invadopodia formation assays.

RESULTS: MDA-MB-231 alpha6 null clones have been generated. Sequencing results show frameshift mutations and deletions of original start codon that all result in premature stop codon and truncated protein product. Alpha6 null clones show no surface alpha6 expression in flow cytometry. Preliminary data show reduced tumorsphere formation ability by alpha6 null compared to alpha6 WT.

CONCLUSIONS: Alpha6 null cells having non-functional alpha6 integrins possess reduced tumorigenic potential. Alpha6 integrin contributes to breast cancer cell tumorigenicity, and the different functional roles of distinct alpha6 integrin cytoplasmic tails are still under evaluation.

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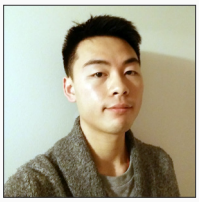

DAVID LU

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- *POSTER SESSION*
- *SUPERVISOR: DR. CATHERINE POH*

HPV-NEGATIVE SMOKERS WITH NEGATIVE P53 EXPRESSION IS ASSOCIATED WITH -3YEAR LOCAL RECURRENCE IN OROPHARYNGEAL CANCER

David Lu[1,2], Kelly Liu[1,2], Rosilene Soares[3], Cindy Cui[2], Jonn Wu[2], Catherine Poh[1,2]

BACKGROUND/OBJECTIVES: The incidence rates of oropharyngeal squamous cell carcinoma (OpSCC) have increased due to the life style change and the prevalence of high-risk human papillomavirus (HR-HPV). It is known that OpSCC patients with positive HR-HPV have better outcome. HPV E6/E7 oncoproteins are known to downregulate p53 and retinoblastoma protein (pRB) expression. However, the biological significance of these markers in OpSCC is not well understood therefore, the main objectives for this study were: 1) to determine the prognostic significance of HPV status, p16, p53 and pRB, smoking history, and treatment for overall survival in OpSCC patients; and 2) to evaluate the biological significance of p53 and pRB expression in relation to HPV status.

METHODS: Retrospectively analyzed OpSCC patients from the BC Cancer Agency that were diagnosed from 2000-2008. Patients with enough formalin-fixed paraffin-embedded (FFPE) oral biopsy tissues (N=234) were included in this study. Serial 5µm sections from constructed tissue microarray (TMA; N=194) or whole section (N=40) from FFPE tissues were subjected to immunohistochemistry (IHC) staining for p16 (E6H4), p53 (DO7), and pRB (Ser807/811). HPV status was determined by combining results of in situ hybridization (ISH) for HR-HPV DNA (types 16/18/31/33/35/39/45/51/52/56/58/59/68) and HR-HPV RNA (types 16/18). Chart reviews were used to collect demographics, smoking history, treatment, and clinical outcomes for 5-year overall survival (OS) and local recurrence (LR). Factors for OS and LR were analyzed by Cox proportional hazard ratios (HR) and Kaplan-Meier survival analysis with log-rank tests ($p<0.05$, two-sided).

RESULTS: Among 234 patients, 76% were male (N=178) and ever-smokers (N=179). Patients' average age was 56.9±9.1 years. All patients were treated with radiotherapy and 42% of patients had combined chemotherapy (N=99). Strikingly, 32% of patients (N=76) died with a median time of 1.6±1.3 years and 14% of patients (N=33) developed LR with a median time of 0.9±0.7 years. Comparing to HR-HPV(-) patients, HR-HPV(+) group (N=180, 77%) had significantly more male and never-smokers with a slightly younger age. Moreover, HR-HPV(+) showed a favourable OS regardless of presence of p53 or pRB. Among ever-smokers (N=179), HR-HPV(+) had a significantly better OS (72% vs. 33%; $p<0.001$). Using multivariate analysis, ever-smokers had a poor OS (HR=3.2, $p<0.01$), which contrasts to combined radio-chemotherapy (HR=0.5, $p<0.05$) and HPV status (HR=0.3, $p<0.001$) for better OS. HPV detection by ISH was relatively better than p16 IHC in OS. Of interest, among ever-smokers who developed LR (N=30), 37% were negative for both HR-HPV and p53 and that was associated with a 3-year LR ($p<0.001$).

CONCLUSIONS: HPV and/or p16 continue to be good prognostic markers in OpSCC. Among HR-HPV(-) and ever-smokers, the absence of p53 expression may be biologically aggressive for the development of LR.

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INVESTIGATING THE EFFECTS OF RUNX1 AND TP53 MUTATIONS ON LENALIDOMIDE RESISTANCE IN DEL(5Q) MYELODYSPLASTIC SYNDROME PATIENTS

Angela Mo[1], Sergio Martinez[1], Aly Karsan[1]

BACKGROUND/OBJECTIVES: Myelodysplastic syndrome (MDS) is a blood cancer characterized by stem cells that cannot differentiate into blood cells. Patients are dependent on blood transfusions, but few treatment options are available. del(5q) MDS is the most common cytogenic subtype of MDS (5-10%), where an interstitial deletion in chromosome 5q render patients sensitive to the drug, lenalidomide (LEN). LEN selectively kills del(5q) cells. However, around 50% of patients relapse within 2-3 years. Little is known about the mechanisms of LEN action and resistance. Our lab obtained whole genome sequencing data for 7 del(5q) MDS patients from before LEN treatment and after relapse. **RUNX1** mutations occurred in 2 patients and **TP53** mutations in another 2 at relapse. RUNX1 is a transcription factor involved in hematopoiesis, and is a known tumor suppressor. TP53 encodes a tumor-suppressor and regulates apoptosis. GeneSet Enrichment Analysis (identifies expression patterns of genes associated with known phenotypes) of paired RNAseq data for 5 of our patients, including the ones with the 2 mutations, indicate a uniform KRAS activation signature at relapse. The GTPase, KRAS, has been implicated in the malignant transformation of many cancers. Based on the mutual exclusivity of the mutations and the uniform KRAS activation signature, we hypothesize that LEN acts through a signaling pathway involving p53 and RUNX1 that converges on KRAS activity.

METHODS: Aim 1- Determine if wild-type p53 and RUNX1 are required for mediating LEN effects on del(5q) cells: We used CRISPR-Cas9 technology to knock out these genes in the LEN-sensitive del(5q) cell line, MDS-L. We treated the cells with LEN in liquid culture and in methylcellulose for CFCs (colony forming cell assay) and compared colony counts. We measured apoptosis by Annexin V staining and flow cytometry analysis. Aim 2- Determine if over-expressing active KRAS can cause LEN resistance: Using lentivirus, we over-expressed GFP-tagged, active KRAS G12V mutant in MDS-L cells and performed the same assays.

RESULTS: We confirmed RUNX1 knock-out (KO) by Sanger sequencing, and no RUNX1 protein is detected by western blot. Based on Annexin V staining and colony formation, RUNX1 KO cells have a reduced LEN response compared to parental cells based on CFC colony counts (2-way ANOVA, p= 0.003) and percentage of Annexin V positive cells (2-way ANOVA, p=0.0002). For our TP53 KO cells, Sanger sequencing indicates that our positive clones have out of frame indel mutations and early stop codons, but p53 protein is still detectable by western. KRAS G12V expressing cells have a reduced response to LEN based on day 7 cell counts (n=2), and they form fewer colonies even at basal levels without LEN. However, Annexin V staining is comparable with control cells with LEN treatment.

CONCLUSIONS: RUNX1 is necessary for mediating LEN effects and KRAS activation may play a role. We will create CRISPR knock-ins emulating the patient mutations, and investigate alternative methods of KRAS activation. Understanding mechanisms of LEN activity can potentially lead to new therapeutic targets and a better understanding of the disease.

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COXSACKIEVIRUS BLOCKS AUTOPHAGIC FLUX THROUGH CLEAVAGE OF SNAP29

Yasir Mohamud[1,2], Junyan Shi[2], Tak Poon[1], Yuan Xue[1,2], Eric Deng[1,2], Jingchun Zhang[1], Honglin Luo[1,2]

BACKGROUND/OBJECTIVES: Coxsackievirus is among the most prevalent etiological agents associated with viral myocarditis, an important cause of cardiovascular mortality worldwide. Autophagy is a fundamental cellular process by which damaged organelles/misfolded proteins are sequestered within autophagosomes and degraded following fusion with lysosomes. We have recently discovered that coxsackievirus infection inhibits the fusion of autophagosomes with lysosomes (autophagic flux). However, the exact mechanism and its functional significance have not been fully elucidated. Here we hypothesize that coxsackievirus inhibits autophagic flux via blockage of SNARE-mediated autophagosome-lysosome fusion, leading to enhanced viral release and disrupted host protein homeostasis.

METHODS & RESULTS: Co-immunoprecipitation study showed that physical interaction between STX17 (autophagosomal SNARE protein) and VAMP8 (lysosomal SNARE protein) was impaired during coxsackievirus infection. Using western blotting, in vitro cleavage assay, and site-directed mutagenesis, we further demonstrated that SNAP29, another SNARE protein that drives autophagosome-lysosome fusion by binding to both STX17 and VAMP8, was cleaved at Q161 following infection through the action of virus-encoded proteinase 3Cpro. Finally, we showed that gene-silencing of SNAP29 resulted in increased viral release.

CONCLUSIONS: We identified a novel mechanism by which coxsackievirus subverts the autophagic pathway to promote viral propagation, and consequently promoting the pathogenesis of disease.

AFFILIATIONS

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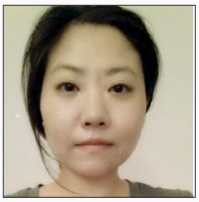

HAISLE MOON

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. JAYACHANDRAN N. KIZHAKKEDATHU*

NOVEL CELL-SURFACE ENGINEERING METHODS TO INCREASE IMMUNE-TOLERANCE OF ALLOGENIC CELL TRANSPLANTATION

Haisle Moon[1,3], Erika Siren[2,3], Jayachandran Kizhakkedathu[1,2,3]

BACKGROUND/OBJECTIVES: Cell surface modified living cells/tissues are used to address the challenges in modern transplantation medicine, especially immunological rejection of donor cells, tissues, and organs. The success of such cell-based therapeutic approaches depends on the ability of modified cells to avoid the immune detection. For example, it has been shown that creating a physical barrier on red blood cell (RBC) surface by grafting voluminous hydrophilic polymers minimize the adverse immune reaction, by modulating the interaction between the host immune system and the cell surface. However, chemical method of conjugating polymers and macromolecules onto cell surface often suffers from instability and toxicities issues. In this study, we address this challenge by developing a novel enzyme mediated (transglutaminase) cell-surface modification method to generate universal donor RBCs. The enzyme mediated polymer grating on living cell membrane is a novel and mild strategy in the surface engineering of cells and can be advantages when compared to chemical approaches. In addition, it can be performed at 4 °C- the storage temperature of RBCs.

METHODS: Highly biocompatible and hydrophilic hyperbranched polyglycerol (HPG) polymers were synthesized and functionalized with glutamine-tagged peptides (GLQQQGNAc). The functionalized HPGs are then characterized by proton NMR to determine the degree of functionalization. To enzymatically graft glutamine-containing peptide substrate functionalized HPGs to amine groups from lysine side chains of RBC surface glycoproteins, simple amide bond formation catalyzed by tranglutaminases (TGases) extracted from guinea pig liver (Sigma-Aldrich, USA) was initiated in presence of 5mM CaCl2 and 3mM glutathione (GSH) in PBS. In addition, enzymatic grafting of HPG on RBCs using TGases is enhanced using a neutral macromolecular crowder, 40kDa dextran (100 – 220mg/mL). To assess the successful grating, the functionalized HPGs are fluorescently labeled and the mean fluorescence intensity is measured by flow cytometer (Beckman Coulter, USA).

RESULTS: From preliminary observation, enzyme mediated polymer grafting on RBC surface using TGases without dextran additive showed an average 2-fold increase in florescent intensity, when compared to RBC control, at room temperature (RT) at 30 mins. Highest increase in fluorescent intensity was observed at RT showing 11.4-fold increase in HPG grafting when the enzymatic reactions are performed in presence of dextran (220mg/mL concentration) and at 4oC showing 13.6-fold increase in fluorescence intensity in presence of dextran (150mg/mL for 2hrs) demonstrating the proof-of-concept.

CONCLUSIONS: Enzyme mediated RBC modifications using a macromolecular crowding is quick, and convenient method to modify the surface of live cells. We anticipate that the method could be adapted to generate antigen protected RBCs in an effort to enhance transfusion safety.

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LORENZ NIERVES

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. PHILIPP LANGE*

OPTIMIZING A PROTEOMIC WORKFLOW FOR THE ISOLATION OF CELL SURFACE PROTEINS AND TERMINI

Lorenz Nierves[1,2] and Philipp Lange[1,2]

BACKGROUND/OBJECTIVES: Recent advances in targeted immunotherapy led to renewed interest in exploring the plasma membrane's (PM) proteome and how changes in this sub-proteome could be linked to many diseases. Additionally, there is strong evidence that deregulated proteases in the microenvironment play an important role in the pathogenesis of cancer. It is therefore possible that the processing of cell surface proteins is altered in cancer. We hypothesize that the altered microenvironment and cancer-specific proteases present in the bone marrow (BM) of Acute Lymphocytic Leukemia (ALL) lead to new and targetable cell surface protein termini that are not found on normal cells.

Objective: To optimize a proteomic workflow that will enable the isolation and analysis of cell surface proteins and termini from limited amounts of clinical samples.

METHOD: To enrich for cell surface proteins and termini, we used two complementary approaches. I) We adapt the well-established cell-surface biotinylation/streptavidin (SA) pull-out to enrich for cell surface proteins and to simulaneously label surface-exposed protein N-termini. Briefly, intact cells were incubated with biotin to label proteins localized at the plasma membrane. After, cells were lysed and the lysate was incubated with magnetic SA beads. Biotin-labeled proteins would then bind to the magnetic SA beads. Positively selected proteins are eluted from the beads and further processed for Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) analysis. II) We combined cell-surface biotinylation with Terminal Amine Isotope Labeling of Substrates (TAILS) to enrich for cell surface termini. TAILS enables enrichment and global assessment of protein termini. For this approach, biotin was used to label and protect accessible cell surface termini and lysine residues. Lysates are then digested and incubated with an amine reactive polymer. Amine (–NH2) groups from N-terminal peptides and non-biotinylated lysine were bound to the polymer and filtered by size cut-off; thereby negatively selecting for the biotinylated termini. As in the previous approach, the termini-enriched samples were further processed for analysis using LC-MS/MS. Using Jurkat (T-ALL cell line) cells, we are currently optimizing the workflow's parameters including the amount of starting material, incubation times, and buffer conditions. Once the workflow is reliably capable of enriching for cell surface proteins and termini from limited amounts of Jurkat cells, we will move on to clinical samples. We have 60 clinical samples from paediatric pre-B ALL and T-ALL patients that are currently stored in the BC Children's Hospital Biobank.

CONCLUSIONS: From this analysis, we will derive a panel of cancer-specific termini to be characterized further. We will validate and investigate the effect of an altered microenvironment and proteases on the synthesis of these cancer-specific termini. To do this, we will alter cell culture conditions and incubate cells with recombinant proteases. The optimized proteomic workflow would then be used to monitor the changes in the termini over time.

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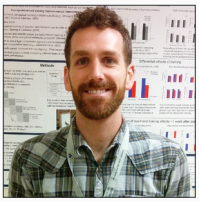

CAMERON ORAM

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. DAVID GRANVILLE*

EXTRACELLULAR GZMB IN SPINAL CORD INJURY AND ASTROCYTE REACTIVITY

Cameron Oram[1,2], Valerio Russo[1,2], Steven Shen[1,2], Christopher Turner[1,2], Matt Zeglinski[1,2], Keerit Tauh[1,2], Stephanie SantaCruz[1,2], Wolfram Tetzlaff[1] and David Granville[1,2]

BACKGROUND/OBJECTIVES: Granzyme B (GzmB) is a serine protease that is well characterized for its role in cytotoxic T-lymphocyte mediated apoptosis. GzmB may contribute to tissue destruction during chronic inflammation through the degradation of extracellular matrix (ECM) proteins. Conditions involving neuroinflammation including multiple sclerosis (MS) and spinal cord injury (SCI) are characterized by immune cell infiltration. These immune cells can contribute to long term tissue damage by inducing apoptosis in neural cells and secreting cytokines and proteases. This chain of events leads to the activation of local astrocytes resulting in the formation of a regeneration-restrictive glial scar. In this preliminary study we hypothesized that GzmB is present in the spinal cord lesion site shortly after injury. In addition, we hypothesized extracellular GzmB induces a reactive phenotype in astrocytes (increased GFAP, ECM expression).

METHODS: C57Bl/6 mice were subjected to a thoracic contusion. Spinal cord lesions were assessed 5 days post-injury and stained for GzmB, immune cell markers and ECM proteins (fibronectin, decorin). Primary astrocytes were isolated from E18 rat cortices through an established differential shaking method. Astrocytes were exposed to GzmB alone and observed by fluorescent microscopy. Cell viability was measured by fluorescent emission in cells to calcein-AM (live) and ethidium homodimer-1 (dead).

RESULTS: GzmB and CD68 positive cells were identified in the lesion site of mice 5 days after injury and corresponded with disrupted neurofilament and glial scar ECM proteins. Primary astrocytes subjected to GzmB underwent apoptosis within 30 mins post-exposure suggesting they are highly susceptible to GzmB.

CONCLUSIONS: These results suggest a negative role for GzmB during SCI that may contribute to glial cell death at early or late stages of injury. Studies are currently ongoing to characterize the knockout and inhibition of GzmB in both short and long term mouse models of SCI.

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JESSICA PILSWORTH

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. DAVID HUNTSMAN*

TERT PROMOTER MUTATION IN ADULT GRANULOSA CELL TUMOURS OF THE OVARY: PREVALENCE AND PROGNOSTIC SIGNIFICANCE

Jessica Pilsworth[1,2], Dawn Cochrane[2,3], Zhouchunyang Xia[2,3], Hugo Horlings[2,3], Winnie Yang[2,3], Melissa McConechy[2,3,4], Satoshi Yanagida[2,3], Anniina Farkkila[5], Adele Wong[6], Genny Trigo-Gonzalez[7], S.W. Grace Cheng[7], Yikan Wang[2,8], Ali Bashashati[2,8], Gregg Morin[1,7], Esther Oliva[6], Sohrab Shah[2,8], David Huntsman[2,3,9]

BACKGROUND/OBJECTIVES: Adult granulosa cell tumours (AGCTs) of the ovary account for 90% of sex cord-stromal tumours and have a high recurrence rate up to 50%. A single somatic missense mutation in the FOXL2 gene (c.402C>G; pC134W) is a defining feature of AGCT and can be used as a robust marker for diagnosis in specific clinical situations. FOXL2 is a forkhead transcription factor that is crucial for proper reproductive function in females. Functional studies investigating the consequences of this FOXL2 mutation have yielded minimal information on its regulatory defects, as the current cell models do not recapitulate the biology of these tumours. Further, no secondary recurrent mutations have been identified and pathogenesis of AGCT remains unknown. Thus, determining additional genetic events in AGCT is essential to understanding and improving prognosis.

In a pilot study, we performed whole genome sequencing on ten AGCTs and matched normal cases to generate a comprehensive catalogue of coding and non-coding events. We identified a TERT promoter mutation (c.228C>T) in 50% of these cases. Previous research has shown that TERT activation is evident in over 90% of cancers and is a fundamental step in tumourigenesis that enables unlimited proliferation. This TERT promoter mutation in AGCT provides an explanation of how granulosa cells escape atresia and attain immortality. Thus, we hypothesize that FOXL2 mutation prevents apoptosis and TERT mutation allows limitless proliferation for transformation of granulosa cells.

METHODS: We assembled a large international extension cohort of approximately 400 cases of AGCTs for validation. An allelic discrimination assay was used to determine the prevalence of TERT (C228T) promoter mutation and Kaplan-Meier analysis was performed to evaluate its prognostic significance. We are also interrogating other sex-cord stromal tumours, including Thecomas, Fibromas, and Sertoli-Leydig cell tumours, to compare the frequency of the TERT (C228T) change across these tumour types.

RESULTS: TERT (C228T) was detected in 26% (82/320) cases in our AGCT extension cohort. In AGCT, we found that this TERT mutation was correlated with a significantly worse survival outcome in patients with primary AGCT ($p<0.005$). Further, we found that TERT (C228T) mutation was present in a larger proportion of recurrent cases.

CONCLUSIONS: We propose that the TERT promoter mutation works in concert with the FOXL2 mutation to transform granulosa cells and may represent a subtype of AGCT with a worse prognosis. We are working to develop more appropriate models to establish biological consequences of the FOXL2 and TERT mutations and identify targets for novel therapeutic strategies.

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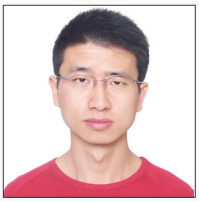

YE QIU

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. DECHENG YANG*

COXSACKIEVIRUS-INDUCED UPREGULATION OF MICRORNA21- PROMOTES CELL APOPTOSIS VIA TARGETING POLY(RC) BINDING PROTEIN 1

Ye Qiu[1,2], Hongxin Shen[2], Mary Zhang[1,2], Xin Ye[1,2] and Decheng Yang[1,2]

BACKGROUND/OBJECTIVES: Coxsackievirus B3 (CVB3) is a predominant pathogen of viral myocarditis, an inflammatory disease of myocardium. CVB3 infection induces massive cell apoptosis, which facilitates viral progeny release is one of the major causes of myocardium damage. MicroRNAs (miRNAs) are small non-coding RNAs regulating protein expression via binding to the specific targeting sites on messenger RNAs (mRNAs), leading to mRNA degradation or translational suppression. Change of miRNA expression level is closely related to various cellular signaling pathways, including apoptosis. Here, we aim to determine the role of miR-21, a microRNA upregulated during CVB3 infection, in CVB3-induced apoptosis.

METHODS: Microarray analysis of miRNA expression profiles was performed using RNAs isolated from CVB3-infected mouse hearts. The miR-21 mimics (21-mic) were tranfected into HeLa cells to increase miR-21 level, and the cell viability was observed by phase-contrast microscopy and quantified by MTS cell viability assay. The target gene of miR-21 was predicted by the online bioinformatic program TargetScan and verified by i) dual luciferase assay using reporters contain wild-type (WT) or mutant (Mut, which was mutated at the predicted binding site of miR-21) 3′UTR of poly(rC) binding protein1 (PCBP1) and ii) detection of the PCBP1 levels after transfecting the cells with 21-mic or the miR-21 inhibitor (21-in). PCBP1 was overexpressed in HeLa cells by plasmid transfection and the downstream signals of PCBP1 and Bcl-2 were detected by Western blotting analysis.

RESULTS: Microarray analysis showed a 2–4 fold increase of miR-21 in CVB3 infected mouse hearts at 7 days post infection (pi) compared with the sham-infected control. Transfection of 21-mic in HeLa cells reduced cell viability by ~40% and enhanced caspase-3 cleavage at 6 hours pi. PCBP1 was predicted as a target gene of miR-21. Correspondingly, dual luciferase assay showed that miR-21 reduced the expression of reporter luciferase with WT PCBP1 3′UTR by ~30% but not that regulated by Mut PCBP1 3′UTR, compared to the control microRNA. Meanwhile, 21-mic decreased PCBP1 protein level by ~30% and 21-in increased it by 50% in both sham- and CVB3-infected cells. Finally, overexpression of PCBP1 decreased the expression of anti-apoptotic gene Bcl-2 and reduced cell viability by ~40% upon CVB3 infection.

CONCLUSIONS: CVB3-induced miR-21 promotes cell apoptosis via targeting PCBP1.

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HARPREET RAI

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. PAMELA HOODLESS*

SOX9 RELATED TRANSCRIPTIONAL NETWORKS IN MITRAL VALVE DISEASE

Harpreet Rai[1,2], Victoria C. Garside[2], Rebecca Cullum[2], Paul J. Hanson[3], Michael A. Seidman[4], Michael F. Allard[1], Pamela Hoodless[1,2,5]

BACKGROUND/OBJECTIVES: Mitral Valve regurgitation is the most commonly diagnosed human valve disease. Aberrant activation of embryonic transcriptional networks is increasingly being appreciated as causal in adult valve diseases. Our lab has recently published on the pivotal role of Sry-box transcription factor 9 (SOX-9) in regulating critical transcription networks in embryonic heart valve development in mice. Following on this, we investigated the full spectrum of transcriptional changes associated with myxomatous mitral valve disease (MMVD) in adults. From these preliminary studies, it is hypothesized that aberrant expression of SOX9 activates TGF beta/BMP and Wnt signaling networks, driving changes in the expression of extracellular matrix components that form the underlying substrate of myxomatous valve degeneration.

METHODS: Eighteen (18) adult human mitral valve specimens were obtained under appropriate human ethics protocols from the Cardiovascular Tissue Registry in the Centre for Heart Lung Innovation (HLI). Valves were characterized by cardiac pathologist as normal, MMVD, or post-inflammatory fibrotic (PIF) mitral valve disease using H&E and Movat's pentachrome staining. SOX9 expression (mRNA and protein) was examined using qRT-PCR and immunofluorescence. Furthermore, three (3) specimens from each condition were analyzed by 3' RNA-seq to assess global gene expression changes in human valve disease. Differential gene expression analysis was assessed using DESeq2.

RESULTS: SOX9 mRNA expression was upregulated in MMVD as compared to normal. Differential gene expression showed 1036 genes altered in MMVD as compared to normal. Bioinformatic analysis identified upregulation of gene pathways traditionally associated with bone and cartilage development and downregulation of gene pathways associated with cardiovascular development and oxidative stress response. Furthermore, multiple components of the TGF beta/BMP and Wnt pathways were altered in MMVD, and some of these were also altered in embryonic murine valves lacking SOX9. Specifically, in MMVD, TGF beta pathway genes BMP4, MSX1, WWP2, and TGFBR1 were all upregulated, while TGFBR2, TGFBR3, and DKK2 (a Wnt inhibitor) were downregulated. The Wnt inhibited cardiac development genes MYH6 and RYR2 were highly downregulated, while FRZB and RSOP2, which have roles in regulating Wnt signaling, were upregulated ($p<0.05$).

CONCLUSIONS: Preliminary data suggest that adult human MMVD is characterised by increased expression of SOX9 and misregulation of components of the TGF beta/BMP and Wnt pathways. Aberrant gene expression patterns will be confirmed using cultures of primary human mitral valvular interstitial cells. Further studies will dissect the contribution of the various components of these pathways to identify specific mechanistic and phenotypic implications, with the hope of identifying both candidate biomarkers for better diagnosis of MMVD and novel therapeutic targets.

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SARA SABERI

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. HÉLÈNE C.F. CÔTÉ*

CONCORDANCE BETWEEN PLASMA COTININE CONCENTRATION AND SMOKING SELF-REPORTING BY PREGNANT WOMEN IN THE CARMA COHORT STUDY

Sara Saberi[1,4], Abhinav Ajaykumar[1], Mayanne MT Zhu[1], Izabelle Gadawski[1], Beheroze Sattha[1], Julie Van Schalkwyk[1,3], Deborah M Money[1,2,3], Hélène Côté[1,3,4], and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

BACKGROUND/OBJECTIVES: Most studies use self-reported smoking data. However, stigma associated with smoking during pregnancy may lead to underreporting of this behavior and possible study bias. Cotinine, a nicotine metabolite with a ~16h half-life in plasma, urine, or saliva, is often used as a biomarker of smoking. We examined the concordance between self-reported smoking and plasma cotinine concentration among pregnant women in the CARMA-PREG cohort study.

METHODS: Cotinine was measured by ELISA in plasma collected between 31 and 38 weeks of gestation. Measures were initially done on a sample of 47 HIV+ and HIV- women. In a subsequent analysis, cotinine plasma levels were measured for an additional 31 women to assess the reproducibility of the initial result. We compared the proportion of cotinine-negative and cotinine-positive with self-reported smoking data collected on the same day as blood collection and reported the concordance.

RESULTS: The self-reported smokers and non-smokers were of similar age, however the self-reported smokers were more likely to be Indigenous/First Nations or White/Caucasian, have low income, and deliver preterm ($p \leq 0.03$). Among the first subset (n=47), 55% reported being non-smokers, 26% reported smoking daily and 4% weekly; 15% reported smoking with unknown frequency. Defining smoking as plasma cotinine ≥5 ng/ml, we observed 90% and 88% concordance between plasma cotinine and self-reported smoking and non-smoking, respectively. Among the second subset (n=31), 58% reported being non-smokers, 35% reported smoking daily, 3% weekly, and 3% who smoked had no frequency data. This time, we observed 92% and 94% concordance between plasma cotinine and self-reported smoking and non-smoking, respectively. Taken together, we observed an overall 91% concordance between plasma cotinine and self-reported data.

CONCLUSIONS: These findings suggest that self-reported smoking is a valid measure for smoking during pregnancy in the CARMA cohort, and do not support underreporting as an important source of bias.

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MARTA SALVADOR ORDOÑO

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. HÉLÈNE C.F. CÔTÉ*

PLACENTA PROGESTERONE LEVELS ARE NEGATIVELY ASSOCIATED WITH PLACENTA MITOCHONDRIAL DNA CONTENT AMONG HIV+ AND HIV- WOMEN IN THE CARMA-PREG COHORT

Marta Salvador[1,4], Izabelle Gadawski[1],Beheroze Sattha[1], Deborah Money[1,2,3,6], Hélène Côté[1,2,4], Isabelle Boucoiran[1,5,6] and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

BACKGROUND/OBJECTIVES: Introduction: Preterm birth (PTB), defined as infants born at <37 weeks of gestation, is the leading cause of death and morbidity among children and was responsible for more than 1 million deaths in 2015. PTB occurs in 6–10% of births among the general population, however among pregnant women living with HIV, the rates are much higher, ranging from 18 to 29%. To date there is no generally accepted mechanism underlying such increased rates. One possible explanation is the reduced maternal progesterone production during pregnancy, which may be related to HIV infection and/or antiretroviral treatment. Synthesis of progesterone is central to pregnancy maintenance and is dependent on placental mitochondrial function. Given that many antiretrovirals can affect mitochondrial (mt) function, we investigated the relationship between placenta mtDNA content, a marker of mt function and placenta progesterone levels, as these may reflect increased risk of preterm delivery.

METHODS: Placental tissue, clinical and sociodemographic data were collected for pregnant women enrolled in the prospective Canadian cohort CARMA-PREG. Placental progesterone levels were measured by ELISA while mtDNA content was quantified by monochrome multiplex qPCR. Univariate associations between progesterone, mtDNA, and preterm delivery (<37w gestation, any etiology) were investigated by Mann-Whitney, Chi2, Pearson's or Spearman's correlation tests.

RESULTS: Placenta tissue was obtained from 136 HIV-infected and 60 HIV-uninfected participants aged 18-45y, of whom 24 (18%) and 9 (15%) had a preterm delivery, respectively. Infants of mothers living with HIV had lower gestational age (p=0.017) and weight (p=0.011) at birth compared to controls. Within this cohort sample, preterm delivery showed no association with HIV status, placenta mtDNA or progesterone. However, placenta mtDNA and progesterone levels were significantly negatively correlated to one another (n=196, rho=-0.242, p<0.0001). In addition, women who delivered by caesarean section (38%) had higher placenta mtDNA than women who had a vaginal delivery (62%, p=0.032).

CONCLUSIONS: Our preliminary results suggest an association between lower levels of progesterone in the placenta and increased levels of mtDNA, possibly reflecting a compensatory mechanism for mitochondrial dysfunction. Placenta mtDNA association with mode of delivery may be related to the stress/energy demand during labour and/or placental insufficiency. Possible associations with preterm delivery etiology (iatrogenic vs spontaneous) and pregnancy complications will be examined once the sample size is larger.

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TSEDAY ZEWDU TEGEGN

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. ED PRYZDIAL*

DENGUE VIRUS INDUCED PROTEOME CHANGES IN PLATELETS

Tseday Zewdu Tegegn

BACKGROUND/OBJECTIVES: According to the World Health Organization, about 50% of the world's population lives in regions endemic to dengue virus (DENV) and are at high risk of infection. Approximately 400 million people are infected yearly, of which about half are asymptomatic despite high virus titers. Transfusion products from these donors often bypass the current screening methods, thereby posing a risk of transmitting DENV by transfusion. In a recent study, our lab demonstrated that DENV can bind and enter platelets, utilizing the simple cellular machinery to replicate and assemble new infectious virus progeny. This helps to explain thrombocytopenia known to be caused by DENV, yet the mechanism is not well understood. Here we will follow the changes in platelet proteome caused by DENV infection. The proteomics data collected will have future implication on antiviral design for DENV prevention. Platelet transfusion quality control can be defined by evaluating critical proteins found to be affected by DENV. Aim 1: Understanding the DENV envelope proteome composition. DENV has an envelope which is a phospholipid bilayer derived from host cell containing virus and host encoded proteins. Understanding the host-derived proteins on the virus surface is of primary importance to identify surface receptors that can be targeted for antiviral drugs. Aim 2: Define the DENV induced platelet proteome. Our lab has shown that platelets produce infectious virus. We expect that the 10 virus-encoded proteins will be present within the platelet proteome after infection. Any changes in the platelet proteome as compared to non-infected platelets would be expected to be induced by DENV. This data can give us insight on the mechanism by which the DENV infection causes thrombocytopenia.

METHODS: Virus propagation and purification will be performed for DENV serotype 2, which is well established in our lab. Epithelial and endothelial cell culture will be utilized to compare host encoded proteins associated with the DENV envelope. Proteomic analysis to identify and characterize key proteins will be done using high performance liquid chromatography coupled to mass spectrometry. Proteins will be metabolically-labeled using stable isotopes and less costly, but non-quantitative, biotinylated puromycin. Proteomics validation will be performed by western blot and immunofluorescence confocal microscopy.

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THYRZA TOLEDO

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. SUZANNE VERCAUTEREN*

CIRCULATING DNA ANALYSIS IN CHILDREN WITH SOLID TUMORS GIVEN GRANULOCYTE COLONY STIMULATING FACTOR

Thyrza Toledo[1,2] and Suzanne Vercauteren[1,2,3]

BACKGROUND/OBJECTIVES: Solid tumors make up about 30% of all pediatric cancers. Children with solid tumors may receive a drug called Granulocyte Colony Stimulating Factor (G-CSF) to treat chemotherapy induced neutropenia or to prepare for stem cell harvest. For the latter, patients will be given G-CSF to stimulate the bone marrow and move stem cells into the blood. These stem cells are then collected and frozen for future infusion. However, recent studies in mice have shown that G-CSF can promote tumor growth and metastasis in vivo. We aim to study whether G-CSF can stimulate tumor growth in solid tumor patients by using a non-invasive method of measuring tumor specific circulating cell-free DNA (cfDNA) in the blood. Also, it is possible that residual tumor may still be present in the bone marrow and may be collected during stem cell harvest. If transplanted back into the patient, this could result in tumor growth and relapse. We aim to study whether the level of tumor specific cfDNA in the plasma product correlates with the level of tumor specific genomic DNA (gDNA) in the stem cell product after G-CSF stimulation. These are important questions to address because this could affect the future use of G-CSF as a drug and provide a non-invasive method to assess the presence of residual tumor.

METHODS: Plasma samples were collected from 11 children with solid tumors before and after G-CSF treatment. Stem cells were also obtained from 6 children with solid tumors. Total cfDNA was measured by quantitative PCR (qPCR) using RNA polymerase II probe. Previous studies have shown that promoter methylation of the Ras Association Domain Family 1 Isoform A gene is common in primary tumors but rare in healthy individuals. Thus, we differentially quantified tumor specific and normal cfDNA by performing methylation specific qPCR.

RESULTS: Out of the 11 children with solid tumors analyzed, six [2 Rhabdomyosarcoma, 3 Neuroblastoma (NB), and 1 Burkitt's lymphoma (BL)] had higher total cfDNA levels post G-CSF while five [4 NB and 1 BL] had lower total cfDNA levels post G-CSF. Although, there was no statistical difference observed in the total cfDNA levels pre and post G-CSF when all 11 children were analyzed ($p=0.8026$). Also, when plasma and stem cells collected at the day of stem cell harvest from 6 children were analyzed, there was no evidence of tumor gDNA contamination in the stem cell product while there was evidence of tumor cfDNA in the plasma product for 4 out of the 6 children. Interestingly, these 4 children were diagnosed with NB and had morphological evidence of metastasis to the bone marrow while the other 2 children were diagnosed with brain tumors, Choroid Plexus Carcinoma and Glioblastoma.

CONCLUSIONS: Tumor specific cfDNA can be detected in the plasma of some children with pediatric cancer at time of stem cell harvest. All children with tumor cfDNA detected had bone marrow metastasis at time of diagnosis, but the presence of tumor specific cfDNA was not indicative of tumor contamination of the stem cell product. Further studies are examining the presence of tumor specific cfDNA before and after G-CSF treatment to see if G-CSF promotes tumor growth.

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KEVIN TSAI

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. JOHN PRIATEL*

DUAL T CELL RECEPTOR-EXPRESSING CD8 T CELLS POTENTIATE AUTOREACTIVITY

Kevin Tsai, Yu-Hsuan Huang, Xiaoxia Wang and John Priatel

BACKGROUND/OBJECTIVES: Central tolerance serves to eliminate newly developing T cells that express strongly autoreactive T cell receptors. Although central tolerance is efficient in deleting high avidity autoreactive T cells, some lower avidity autoreactive T cells escape negative selection to cause autoimmune diseases. Although tight allelic exclusion limits thymocytes to expressing a single TCRbeta chain, rearrangement of the TCRalpha chain continues unabated until halted by positive selection, enabling thymocytes to express up to two TCRalpha chains and thus two TCRs. Moreover, it has been postulated that pathogenic low avidity autoreactive CD8 T cells may escape central tolerance through expression of a secondary benign TCR that mediates positive selection. To determine the role of dual TCR expressing CD8 T cells in autoreactivity and autoimmunity, we have compared CD8 T cell autoreactivity against the model autoantigen ovalbumin between T cells capable of expressing two TCRs (TCRalpha+/+) versus T cells capable of expressing a single TCR (TCRalpha+/-). TCRalpha+/- CD8 T cells exhibited reduced proliferative capacity upon OVA stimulation relative to TCRalpha+/+ CD8 T cells.

In addition, a lower frequency of TCRalpha+/- CD8 T cell effectors produced IFN-gamma upon activation with OVA compared to TCRalpha+/+ CD8 T cell effectors. Taken together, we shown that dual TCR expression by CD8 T cells reduces the efficiency of T cell tolerance and may potentiate T cell autoreactivity. We are investigating whether dual TCR-expressing T cells are key to the pathogenesis of autoimmune diabetes in our mouse model system. Our results will provide valuable insight into the escape mechanisms exploited by pathogenic autoreactive CD8 T cells.

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SREEPARNA VAPPALA

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INHIBITION OF PROTHROMBOTIC CELL-FREE DNA AND NEUTROPHIL EXTRACELLULAR TRAPS WITH POLYCATIONIC INHIBITORS

Sreeparna Vappala[1]

BACKGROUND/OBJECTIVES: Thrombosis is a common pathology underlying ischemic heart disease, ischemic stroke, venous thromboembolism and is invariably associated with sepsis. It is the leading cause of mortality worldwide. Although current drugs are very effective in reducing thrombosis, bleeding is the major side effect associated with their use. These drugs target key players in the coagulation cascade resulting in a hemostatic imbalance favoring bleeding. Recently, important mediators of pathological thrombosis have been identified, including cell-free DNA and Neutrophil extracellular traps (NETs). NETs are released from neutrophils when activated by microbial or inflammatory stimuli. These web like structures are composed of DNA, histones and antimicrobial proteins. They trap and kill microorganisms, playing a critical role in host defense. NETs are extensively produced during condition of sepsis to combat overwhelming bacteremia. Studies have shown that, NETs not only entrap and clear pathogens but also, activate procoagulant factors initiating thrombosis. My objective is to develop polymeric inhibitors with optimized cationic charge density and binding constants to selectively bind and neutralize NETs and cell-free DNA in in vitro, ex-vivo and in mouse models of sepsis. The hypothesis is that, newly designed inhibitors could be used to target and inhibit prothrombotic actions of cell-free DNA and NETs without the risk of bleeding.

METHODS: Neutrophils isolated from human blood will be activated with phorbol-12-myristate-13-acetate to release NETs. Cell-free DNA can be obtained from the Genomic DNA isolated from mammalian fibroblasts or blood. I will perform plate based coagulation assay, thrombin generation assay and thromboelastometry to screen the polymeric inhibitors reversing the prothrombotic action of NETs and cell-free DNA. Optimal candidates from this screening will be used for further binding studies and ex-vivo studies. Furthermore, I will investigate the potential of these molecules to prevent thrombosis in mouse cecal ligation and puncture model –in vivo model for sepsis. Potential candidates will be further studied for their pharmacokinetic profile and tolerance in mice.

RESULTS: From thromboelastometry experiment, the clotting time of the whole blood was found to be less when it was spiked with 100ng/ul of genomic DNA compared to the buffer control. This is congruent with the previous results indicating the prothrombotic nature of genomic DNA. I have optimized the plate based coagulation assay and thrombin generation assay to screen the potential polymeric inhibitors. With these assays, I will be able to identify the polymeric inhibitors specifically neutralizing NETs and cell-free DNA. It is expected that, optimal candidates will bind to the target with high affinity and prevent thrombosis in mouse sepsis model.

CONCLUSIONS: Current antithrombotic therapies are not optimal and has a major side effect of bleeding which could be life threatening. Our approach to target cell-free DNA and NETs –recently identified mediators of thrombosis will have the potential to reverse thrombosis and minimize risk of bleeding.

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BRENNAN WADSWORTH

- *GRADUATE STUDENT*
- *POSTER SESSION*
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2-18F-FLUOROETHANOL IS A NOVEL POSITRON EMISSION TOMOGRAPHY (PET) REPORTER OF SOLID TUMOUR PERFUSION

Brennan Wadsworth[1,2], Jinhe Pan[3], Iulia Dude[3], Nadine Colpo[3], Momir Bosiljcic[1,2], Kuo-Shyan Lin[3], Francois Benard[3], Kevin L Bennewith[1,2]

BACKGROUND/OBJECTIVES: Solid tumour perfusion is a proven variable of interest for predicting cancer aggression and response to therapy. Current methods for non-invasively imaging tumour perfusion with positron emission tomography (PET) are limited by restricted accessibility and short half-lives of perfusion radiotracers. This study presents 2-^{18}F-fluoroethanol (2-^{18}F-FEtOH) as a perfusion reporter that can distinguish between tumours of varying perfusion levels and can be applied to screening drugs that modify tumour perfusion.

METHODS: Uptake of 2-^{18}F-FEtOH in 4T1 and 67NR murine mammary carcinoma tumours grown in mice was measured using ex vivo radiography as well as static and dynamic PET imaging. 2-^{18}F-FEtOH uptake was directly compared with the 14C-Iodoantipyrine perfusion reporter and perfusion-modifying drugs nicotinamide, pentoxifylline, and hydralazine were utilized to manipulate tumour perfusion prior to 2-^{18}F-FEtOH injection.

RESULTS: Uptake of 2-^{18}F-FEtOH in 4T1 and 67NR tumours was consistent with known perfusion differences within and between these tumours. 2-^{18}F-FEtOH uptake correlated well with 14C-Iodoantipyrine, and reflected the anticipated tumour perfusion modifying effects of each drug tested. Patlak modelling of radiotracer uptake supports that differences in 2-^{18}F-FEtOH uptake were dependent on perfusion and not cellular ability to metabolize FEtOH.

CONCLUSIONS: 2-^{18}F-FEtOH is a novel 18F-based radiotracer for investigating tumour perfusion with PET imaging. Quantification of 2-^{18}F-FEtOH uptake can be used to distinguish between tumours of varying perfusion, and to screen the efficacy of blood flow modifying drugs for use as adjuvants to existing cancer therapies.

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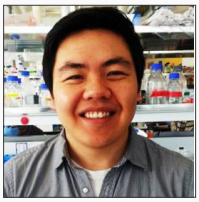

CHRIS WANG

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. MADS DAUGAARD*

INTERNALIZATION MECHANISM OF MALARIA PARASITE PROTEIN INTO CANCER CELLS

Nader al Nakouzi[1,3], Desmond Hui[1], Chris Kedong Wang[1,2], Htoo Oo[1,3], Jamie Rich[4], John Babcock[4], ALi Salanti[5], & Mads Daugaard[1,2,3]

BACKGROUND/OBJECTIVES: The limited clinical efficacy of anti-cancer drugs is a direct consequence of their non-selective nature. In order to improve their efficacy, targeted therapy approaches are needed. One of the major strategies is to conjugate drugs to a delivery vehicle that displays selective binding to tumor cells. Our lab seeks to advance a novel targeting approach, taking advantage of a malaria protein (rVAR2) that binds with high affinity to oncofetal chondroitin sulfate A (ofCSA) specifically present on the placenta and cancer cells. In addition to binding to cancer cells, rVAR2 is internalized into the cell. Internalization is complicated process and depends on the nature of the antigen. Different mechanisms of internalization that could be involved are clathrin and caveolae mediated endocytosis, as well as macropinocytosis. Once in the cell, the release of the drug payload from the protein is dependent on the cellular compartment (affected by pH, Proteases etc.). As such it is equally important to study the intracellular trafficking of rVAR2 in cells. Data from this project will build a foundation for future designs of rVAR2-drug conjugates that will maximize drug efficacy and reduce toxicity.

METHODS: Different cancer cell lines were incubated with rVAR2 at different time points and was investigated by confocal, live cell, and electron microscopy as well as western blotting. Specific fluorescent dyes, tracers, and antibodies were used to determine the intracellular localization of rVAR2. Inhibitors of internalization pathways was also used to determine the route of entry.

RESULTS: Our data show that rVAR2 binds different cancer cells within 5 min and gets rapidly internalized into intracellular vesicular structures. Furthermore, rVAR2 is internalized through micropinocytosis and not clathrin or caveolin mediated. Inside the cells, rVAR2 is localized to the early endosome within 2hrs, however it does not reach the lysosome after 4hrs.

CONCLUSIONS: Due to its ability to specifically target cancer cells, rVAR2 is a novel malaria protein that can be exploited as a therapeutic tool against cancer. In order to maximize rVAR2's potential as a delivery tool, we elucidated its internalization mechanism and showed that rVAR2 is actively internalized into cancer cells via micropinocytosis. However, its intracellular trafficking remains to be seen.

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DEREK WONG

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. STEPHEN YIP*

LOSS OF CAPICUA DYSREGULATES ATXN1L

Derek Wong[1] and Stephen Yip[1]

BACKGROUND/OBJECTIVES: Oligodendroglioma (ODG) is a subtype of brain cancer marked by unique clinical and genetic characteristics such as treatment sensitivity and mutations in the ***Capicua*** (CIC) gene. The high frequency of CIC mutations suggests that loss or altered function of the CIC protein may be crucially associated with the good prognosis of ODG. Little is currently known about mammalian CIC. However, in Drosophila, it is a transcriptional repressor regulated by receptor tyrosine kinase (RTK) activity such as EGFR. Previous research in a different disease context, neurodegeneration, has suggested the interaction between CIC and ATXN1L, an interacting partner, to be important for disease progression; however, their relationship and role in brain tumour pathology has yet to be elucidated. We hypothesize that loss of CIC also dysregulates ATXN1L's normal function resulting in aberrant gene expression.

METHODS: CIC knockout (KO) cell lines were established using CRISPR-Cas9 gene editing technique in human embryonic kidney (HEK293), normal human astrocyte (NHA) and human oligodendroglial (HOG) cell lines. ATXN1L localization was assessed by immunofluorescence and western blot. Expression of genes downstream of notch signaling was assessed using real time quantitative PCR in CIC-KO cell lines.

RESULTS: CRISPR-Cas9 treatment of cell lines resulted in stable cell lines with no detectable CIC expression on western blot. Cellular fractionation and immunofluorescence showed exclusion of ATXN1L from the nucleus in CIC-KO cell lines compared to CIC wildtype cell lines. Upregulation of Hes5, roughly two fold ($p<0.05$, Student's t-test) was seen in CIC-KO cell lines while downregulation of ***Hey1*** ($p<0.05$, Student's t-test) was seen in CIC-KO cell lines.

CONCLUSIONS: CIC and ATXN1L have previously been established as physical interactors important in the regulation of CIC target genes. However, the loss of CIC in cancers such as ODG may also result in both the dysregulation of CIC and ATXN1L. Using cell line models, loss of CIC results in the exclusion of ATXN1L from the nucleus as well as partial dysregulation of the notch signaling pathway, which ATXN1L is implicated as a regulator of.

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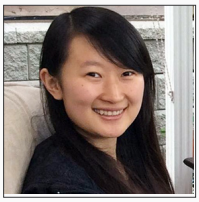

ZHOUCHUNYANG XIA

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. DAVID HUNTSMAN*

USING RETROTRANSPOSONS TO TRACK OVARIAN CANCER DEVELOPMENT

Zhouchunyang Xia[1], Dawn Cochrane[1,2], Michael S Anglesio[1,2], Tayyebeh Nazeran[1], Janine Senz[1], Amy Lum[1], Miguel Alcaide[3], Ali Bashashati[2], Yi Kan Wang[2], Ryan Morin[3], Sohrab Shah[1,2], David G Huntsman[1,2]

BACKGROUND/OBJECTIVES: Women with endometriosis, a painful condition caused by the displacement of tissues that normally lines the uterus, have a three-fold increased risk of developing endometrioid ovarian cancer (ENOC) and clear cell ovarian cancer (CCOC). How two distinct cancers arise from the same precursor lesion is unknown. Sensitive biomarkers are needed to identify women with endometriosis who are at risk of developing cancer. As protein-coding mutations implicated in this malignant transformation only occur in a subset of the patients, additional types of biomarkers are required. We performed whole genome sequencing (WGS) on 29 ENOC and 36 CCOC cases and observed a highly frequent insertion event originating from an active LINE-1 (L1) retrotransposon in the TTC28 gene. L1 retrotransposons are mobile genetic elements that can take downstream DNA pieces and insert them into random genomic locations in a process called 3' transduction. L1s are epigenetically silenced in normal tissues, but are activated in a variety of cancers. We hypothesize that TTC28 L1 retrotransposon is an early event in the transformation of endometriosis into ENOC and CCOC and such events could be used as biomarkers for endometriosis with high cancer risk.

METHODS: We assessed the presence of TTC28 L1 retrotransposition in six different anatomical sites (five tumor and one normal) for four ENOC and four CCOC cases, and compared the results to the presence of six single nucleotide variants (SNVs) and frame shifts mutations in the same tissues. PCR, followed by Sanger sequencing was used to detect TTC28 L1 retrotranspositions, and a micro-fluidic PCR assay followed by MiSeq sequencing was used to detect SNV/frameshift mutations. We developed a target capture sequencing method to identify novel TTC28 L1 retrotranspositions. Oligonucleotide probes tiling 1 kb downstream of L1s are used to capture DNA fragments containing the 3' transductions, and the fragments are sequenced on the MiSeq. Two ENOC and two CCOC cases from the above cohort were tested. Data were analyzed using the R-based software Geneious.

RESULTS: TTC28 L1 retrotransposition events were present at all five tumor sites in 75% (6/8) of cases, while some SNV/frameshift mutations were either absent or were present at varying allelic frequencies. In the remaining two cases, neither TTC28 nor SNV/frameshift mutations were detected in at least one of the tumor sites. Target capture sequencing validated the retrotransposition events identified in WGS for the four cases tested.

CONCLUSIONS: TTC28 L1 retrotranspositions occur early in ovarian cancer tumorigenesis, and such events may reflect the pre-malignant transformation of ENOC and CCOC, and should be explored as a method to track tumor development and evolution. The development of a target capture assay to detect novel L1 transductions is crucial for investigating cases without WGS data. Ultimately, we hope to detect L1 insertions in plasma samples, and use L1 insertions as a biomarker to identify high-risk endometriosis cases.

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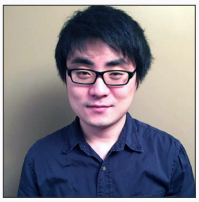

YUAN CHAO XUE

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. HONGLIN LUO*

ENTEROVIRAL INFECTION LEADS TO PROTEIN MISFOLDING AND NEURODEGENERATION

Yuan Chao Xue[1,2], Gabriel Fung[1,2], Yasir Mohamud[1,2], Eric Deng[1,2], Jingchun Zhang[1,2], Ralph Feuer[3], Honglin Luo[1,2]

BACKGROUND/OBJECTIVES: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that targets the motor neurons in the brain and spinal cord, which control the motor movements of the body. The disease is present in similar proportions in the majority of ethnic groups around the world, with the male being the more likely gender to contract the disease. Currently, without any effective therapies, the destruction of the motor neurons will first lead to paralysis, and eventually death.

Even though 5% of all ALS cases have been associated with inherited genetic mutations that have been categorized as familial ALS, the majority of all ALS cases are actually sporadic (95%). In other words, these cases occur in the absence of prior ALS history in the family. Enterovirus (EV), a family of positive-stranded RNA viruses including poliovirus and coxsackievirus, is suspected to influence ALS pathogenesis due to the viruses' ability to target motor neurons. In addition, it has also been shown that patients with prior poliomyelitis, paralysis caused by poliovirus, are at a higher risk of ALS than those without. Our lab recently found that in vitro EV infection results in protein aggregation, RNA-processing defects and disruption of autophagy via EV-encoded proteases. Of particular interest was the finding that EV infection is able to impair nucleocytoplasmic trafficking, and initiate cytoplasmic aggregation and cleavage of transactive response DNA binding protein-43 (TDP-43), one of the hallmarks of ALS. Together with these findings, we hypothesize that EV infection is a causative and/or risk factor in the development of sporadic amyotrophic lateral sclerosis.

METHODS & RESULTS: Neonatal BALB/C mice were infected intracranially with eGFP-coxsackievirus or mock (DMEM) infected. Brain tissues were then collected at 2, 5, 10, 30 days post-infection for performing H&E and immunohistochemical staining. Based on our preliminary data, we were able to show brain lesions and inflammation, identified using IBA1 (microglia), pSTAT3 (astrogliosis) and GFAP (reactive astrocytes) in the cortical and hippocampus regions in parallel with viral protein detection through GFP staining as early as 2 days post-infection. Even though the viral protein was significantly decreased to only 10% of the original intensity at 30 days post-infection, there were sustained inflammatory and immune responses at the later time points. Most notably, our pilot data demonstrated clear ALS-like pathologies, such as cytoplasmic mislocalization and nuclear downregulation of TDP43 at the areas of infection/tissue damages starting at 5 days post-infection and maintained until 30 days post-infection.

CONCLUSION: Our preliminary results reveal that enterovirus infection, such as coxsackievirus, is able to cause ALS-like pathology, especially in the case of TDP-43 abnormality, in virus-infected mouse brains.

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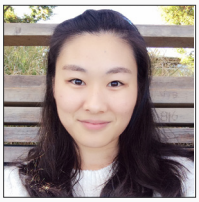

XINING (LINDA) YANG

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. MARK SCOTT*

IA1: A NOVEL PRO-INFLAMMATORY BIOREACTOR IMMUNOTHERAPY

Xining (Linda) Yang[1,3], Wendy Toyofuku[2,3] and Mark Scott[1,2,3]

BACKGROUND/OBJECTIVES: The immune system eliminates tumors through T cell mediated responses. However, when the immune response is compromised, tumors may evade the immunosurveillance. Immunotherapies were developed to boost the activation of T effector cells (Teff). Teff activation can also be achieved through histoincompatibility. Indeed, potential synergy between the MHC-alloresponse and anti-tumor response has been suggested. However, current allostimulatory approaches to treating tumors are dangerous because of the MHC-mismatch. To eliminate the problematic allogeneic cells, we have manufactured a novel cell-free inflammatory agent IA1 from in vitro allorecognition reactions. I hypothesize that IA1 will promote a pro-inflammatory Teff response capable of attenuating tumor cell growth.

METHODS: Human T cell proliferation (CD3+, CD8+ and CD4+) and CD4+-subset differentiation (Th1, Th17 and Treg) were measured via flow cytometry after in vitro (±) IA1 treatment. The control and IA1-activated PBMC were then overlaid on HeLa cells to assess their anti-HeLa proliferation effects using an ACEA iCELLigence instrument.

RESULTS: IA1 promoted a pro-inflammatory proliferation of naïve human lymphocytes. In vitro, a significant ($p < 0.01$) increase in CD3+ cell proliferation was induced. This increase encompassed both CD8+ (cytotoxic T cells: CTL) and CD4+ cells. Furthermore, IA1 maximized the Teff response by increasing the population of the pro-inflammatory Th1 and Th17 while concomitantly decreasing tolerogenic Tregs. IA1 also exerted a synergistic effect on the exiting inflammatory responses in MLR by increasing the T cell proliferation ($p < 0.01$); predominantly by enhancing the CTL and Th17 response. Importantly, IA1 activated naïve lymphocytes significantly inhibited the growth and enhanced the killing of HeLa cells relative to untreated PBMC.

CONCLUSIONS: Bioreactor produced IA1 exerted a pro-inflammatory effect on T cell proliferation and subset differentiation similar to allostimulation. IA1 significantly enhanced the pro-inflammatory response of treated lymphocytes and effectively attenuated tumor cell proliferation.

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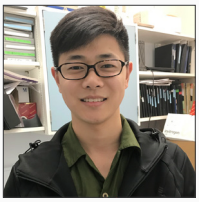

GUANGZE ZHAO

- *GRADUATE STUDENT*
- *POSTER SESSION*
- *SUPERVISOR: DR. DECHENG YANG*

COXSACKIEVIRUS-B-3INDUCED REDUCTION OF INTERCALATED DISK COMPONENT PROTEINS IN VIRAL PATHOGENESIS

Guangze Zhao, Mary Zhang, Ye Qiu, Hua Wang and Decheng Yang

BACKGROUND/OBJECTIVES: Myocarditis is an inflammatory disease of the heart muscle, which is a major cause of sudden death in children and young patients. Among various causes of this disease, viral infection plays the most important role in the pathogenesis, and coxsackievirus B3 (CVB3) is one of the most common pathogens for viral myocarditis. However, the mechanism by which CVB3 induces cardiac damage has not been fully elucidated. Particularly, the involvement of microRNAs (miRNAs), a group of small RNAs regulating the gene expression and subsequent progression of a wide range of diseases, in CVB3 infection is still unclear. Intercalated disks (ICDs) are substantial connections maintaining cardiac structure and mediating signal communication among cardiomyocytes. Deficiency in ICD components leads to heart dysfunction. Our previous research showed that miR-21 is increased by CVB3 infection in cardiomyocytes and we further demonstrated that CVB3-induced miR-21 expression triggers cardiac damage by interfering with ICD structure. In the experiments, we discovered that several important proteins within ICDs decreased during CVB3 infection and some of them were reduced even more upon miR-21 treatment at the same time. For instance, alpha-E-catenin were decreased by both CVB3 infection and miR-21 transfection. However, gamma-catenin (plakoglobin) was decreased in CVB3 infection but not by miR-21 treatment; however. Nevertheless, the mechanisms underlying the decrease of these proteins have not been fully understood. Thus, in this study, we continue to unravel the mysteries of ICD disruption in CVB3 infection via focusing on the regulation of these ICD proteins. Hypothesis: In CVB3 infection, gamma-catenin is decreased by transcriptional inhibition, viral-protease-mediated cleavage or degradation, while alpha-E-catenin is decreased due to upregulation of miR-21, which contributes directly or indirectly to ICDs destruction and development of myocarditis.

METHODS: We used western blot to detect the protein level and potential cleavage products of gamma-catenin and alpha-E-catenin in HeLa cells infected with CVB3. Also, proteasome inhibitor MG132 was used to treat HeLa cells infected with CVB3, and then we compare the western blot results of treated group with that of untreated group to determine the role of protein degradation in the decrease of these two proteins. Meanwhile, cellular RNAs were extracted from CVB3 infected HeLa cells, and real time q-PCR analysis was performed to detect the expression of gamma-catenin and alpha-E-catenin at the transcriptional level.

RESULTS: 1: No cleavage bands of gamma-catenin or alpha-E-catenin were detected in the cell lysis after CVB3 infection; 2: After treating the cells with MG132, the expression level of both alpha-E-catenin and gamma-catenin were increased in both sham- and CVB3-infected cells. 3. The mRNA levels of alpha-E-catenin in CVB3 infected cells showed no significant difference compared to the uninfected cells.

CONCLUSIONS: Decreased protein levels of alpha-E-catenin and gamma-catenin in CVB3 infection are likely due to proteasome-mediated degradation.

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YU-HSUAN HUANG

- *POSTDOCTORAL FELLOW*
- *POSTER SESSION*
- *SUPERVISOR: DR. JOHN J PRIATEL*

THE ROLE OF SAP AND 2B4 CD48/INTERACTION IN B CELL IMMUNOSURVEILLANCE BY EFFECTOR CD8 T CELLS

Yu-Hsuan Huang[1,2], Kevin Tsai[1,2], Sohyeong Kang[1,2], Mandy Ford[3], Marc Horwitz[4] and John Priatel[1,2]

BACKGROUND/OBJECTIVES: X-linked lymphoproliferative disease (XLP) is a rare primary immunodeficiency disease defined by exquisite sensitivity to the B-lymphotropic Epstein–Barr virus (EBV) and is caused by inactivating mutations affecting SLAM-associated protein (SAP). SAP functions as an intracellular adaptor through association with surface SLAM family receptors and has been implicated in regulating lymphocyte/lymphocyte interactions. However, the precise mechanism of how the loss of SAP function contributes to extreme vulnerability to EBV remains unclear. Here, we hypothesize that SAP is critical for effector CD8 T cell cytotoxic functions against virus-infected B cells.

METHODS: Here, we have investigated the cytotoxicity of WT and SAP-deficient CD8 T cells towards various wild type targets. In addition, we have explored the roles of various SLAM family receptors in regulating CD8 T cell cytotoxicity through the use of SLAM family receptor-deficient CD8 T cells and target cells.

RESULTS: SAP- and 2B4- deficient CD8 T cells exhibited impaired cytotoxicity relative to wild type against B cells but not other types of targets. Further, CD48 on the B cell surface is crucial for initiating 2B4-SAP-dependent signaling required for recognition and killing of B cell targets by effector CD8 T cells.

CONCLUSIONS: Altogether, we find that SAP and SLAM family receptor interactions between 2B4 and CD48 are critical for CD8 T cell cytotoxicity against B cell targets and these results help explain the selective immune deficiency of XLP patients towards EBV.

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CANCER ASSOCIATED MUTATIONS IN NON-CANCER ASSOCIATED ENDOMETRIOSIS

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BACKGROUND/OBJECTIVES: Endometriosis affects approximately 10% of reproductive aged women and is characterized by the ectopic presence of functional endometrial tissue (endometrial glands and stroma) outside of the uterus, typically elsewhere in the pelvic cavity. It is a major cause of pelvic pain and infertility. Endometriosis lesions are anatomically classified as either superficial peritoneal endometriosis, ovarian endometriomas, or deep-infiltrating endometriosis (DIE). Endometriosis is considered as a benign inflammatory lesion, but has cancer-like features such as local invasion and resistance to apoptosis. Endometriosis is associated with a 2-4 fold increased risk for endometrioid ovarian cancer (ENOC) and clear cell ovarian cancers (CCOC) .Several studies have reported molecular abnormalities that are shared between ovarian carcinomas and adjacent atypical endometriosis, such as patterns of loss of heterozygosity and mutations in the ARID1A and PIK3CA genes. In this study by using next generation sequencing and digital genomic assays, we demonstrated oncogenic mutations in none cancer associated deep infiltrative endometriosis.

METHODS: Three independent cohorts of deeply infiltrating endometriosis were analyzed. All samples were collected using tissue coring ,macrodissection or Laser capture microdissection from formalin or molecular fixed paraffin embedded archival blocks. Exome-wide (n=24) or cancer driver targeted sequencing (n=3) was performed in 27 discrete deeply infiltrating endometriotic lesions. Mutations were validated using digital-genomics in micro-dissected epithelium and stroma. Epithelial and stromal components from an additional 12 cases were analyzed by droplet digital PCR for recurrent activating KRAS mutations.

RESULTS: A total of 39 cases of non-ovarian deep infiltrating endometriosis lesions were studied from women with an average age of 37 years. Exome sequencing revealed somatic mutations in 20 (83%) of 24 cases. Five lesions harbored somatic mutations in ARID1A, PIK3CA, KRAS, or PPP2R1A, cancer driver-genes that are frequently mutated in ovarian clear cell carcinomas and endometrioid carcinomas. The results were validated by SafeSeqS or immunohistochemistry. The likelihood of affecting driver genes at this rate in the absence of selection was estimated at P= 0.0018 (binomial test). Targeted sequencing and droplet digital PCR uncovered KRAS mutations in 5 of 15 additional cases, with mutations only in endometriotic epithelium, but not stroma. One case harbored two different KRAS mutations, c.35G>T(pG12V) and c.35G>C(pG12A), while another case carried identical KRAS mutation c.35G>A(pG12D) in three distinct lesions.

CONCLUSIONS: We provide evidence that benign deep infiltrating endometriosis harbors somatic cancer driver mutations. Ten (26%) of 39 deep infiltrating lesions carried driver mutations, all confined to epithelium. Our unexpected findings suggest a new research direction to study the pathogenesis of endometriosis and may lead to the generation of a biologically informed classification to ultimately improve prognostication and personalized treatment.

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BARTHOLIN GLAND CARCINOMA :P16 EXPRESSION AND CLINICAL OUTCOME

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BACKGROUND/OBJECTIVES: Primary carcinomas of Bartholin gland are very rare and account for 1% of female genital malignancy and 2-7% of vulvar carcinomas. There are several types of bartholin gland carcinoma (BGC). Adenocarcinoma and squamous cell carcinoma (SCC) are the most common and account for 80%. Bartholin gland carcinomas occur commonly in postmenopausal women but at a younger age compared with non-Bartholin gland related vulvar carcinomas. The pathogenic role of high-risk human papilloma virus (HPV) in squamous cell carcinomas of the female genital tract is well established. It is unclear what proportion of BGCs are associated with high risk HPV infection.

METHODS: We reviewed our hospital archives for all cases of Bartholin gland carcinoma from 1984 to 2017 (N=15). We excluded 3 adenoid cystic carcinomas which were the subject of a previous study. We stained representative slides of 11 available cases for p16 as a surrogate biomarker for high-risk HPV and obtained Clinical outcome information.

RESULTS: There were 11 squamous cell carcinomas (SCC), including one mixed SCC and transitional cell carcinoma and one papillary SCC, and one poorly differentiated adenocarcinoma. Except for one well differentiated keratinizing SCC all tumors were moderately to poorly differentiated.p16 immunostaining was performed on 11 tumors and 10 SCC expressed p16 diffusely and intensely consistent with the presence of HPV, while the single case of adenocarcinoma showed patchy staining. Most of tumors were pathological stage 1b and two were stage 3a with lymph node metastasis . All patients were treated with radical vulvectomy and unilateral or bilateral inguinofemoral lymphadenectomy . Nine cases underwent postoperative radiation therapy. The mean clinical follow up was 53.7 months. Eight patients were free of disease, and recurrence occurred in three cases, with death due to disease in two of the patients with recurrence, including the single patient in this series with adenocarcinoma.

CONCLUSIONS: All SCC of Bartholin gland expressed p16 diffusely and intensely regardless of histologic features and differentiation. Our results and other studies support the etiologic role of HPV in the pathogenesis of SCC of Bartholin gland. In this small study we observed early stage at diagnosis and overall favorable outcomes.

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GRANZYME B: AN IMPORTANT CONTRIBUTOR TO DERMAL-EPIDERMAL SEPARATION IN BLISTERING SKIN CONDITIONS

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BACKGROUND/OBJECTIVES: Blistering skin disorders are a group of skin diseases with various etiologies that involve a loss of epidermal adhesion. In healthy skin, the epidermis and dermis are anchored together at the dermal-epidermal junction (DEJ), a specialized basement membrane pivotal for the integrity and function of the skin. Granzyme B (GzmB) is a serine protease secreted by immune cells that can cleave extracellular matrix proteins. Although elevated GzmB is observed at the DEJ in a number of autoimmune skin conditions, it is unclear as to whether GzmB contributes to such pathologies. Our hypothesis is that GzmB directly cleaves components of the DEJ, thus causing epidermal detachment from the dermis.

METHODS: Paraffin-embedded human skin sections were assessed for GzmB using immunohistochemistry (IHC). Seeking to investigate a potential role for GzmB in mediating cleavage of important components of the DEJ, a cleavage assay was performed on Collagen VII, alfa6beta4 integrin and Nidogen-2. Furthermore, in order to define specific GzmB cleavage sites, a mass spectrometry analysis based on terminal amine isotopic labelling (TAILS) was also performed on these proteins. Finally, samples of healthy skin were exposed to GzmB to investigate in vitro DEJ cleavage through H&E and IHC staining.

RESULTS: GzmB was abundantly expressed in the DEJ of diseased human skin compared to healthy (non-blistering) skin. Epidermal separation from the dermis was observed in healthy skin upon exposure to GzmB. Western blot analysis confirmed GzmB-mediated cleavage of Collagen VII, alfa6beta4 integrin and Nidogen-2, which was prevented by GzmB inhibition. Furthermore, TAILS analysis revealed that Collagen VII is cleaved in a region crucial for its anchoring function.

CONCLUSIONS: Overall our data suggest that GzmB could be responsible for epidermal detachment observed in blistering skin conditions and thus represents a potential target for the prevention of skin blistering.

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QUANTIFICATION OF C-REACTIVE PROTEIN IN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Junyan Shi[1], Emma Zheng[1], Mari De Marco[1,2]

BACKGROUND/OBJECTIVES: C-reactive protein (CRP) in plasma is an acute-phase biomarker of inflammation. CRP concentration provides useful diagnostic information, as well as prognostic information on treatment efficacy for a wide variety of diseases. Due to its broad utility there is constant demand for measurement of CRP in clinical research studies. Current quantitative analysis of CRP in clinical labs relies on immunoassay and upwards of 150 microliter of plasma is needed for analysis. For many studies, utilizing such a large sample volume for a single analyte measurement is impractical given the scope of analyses desired. Therefore, we sought to develop a novel technique to accurately and precisely measure CRP in plasma with minimal volume requirements.

METHODS: High performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was utilized. As an internal standard, stable-isotope-labeled CRP tryptic peptides were spiked into all samples. Samples were denatured, digested and analyzed by HPLC-MS/MS. Absolute quantification was performed by comparison to an external calibration curve. A method comparison was performed against the immunometric method used at St Paul's Hospital.

RESULTS: Using 10 microliter of plasma, the detection limit has been tested down to 2 mg/L. The method was linear from 2 to 80 mg/L (R^2=0.99) and testing of the full reportable range is underway. There was excellent correlation between HPLC-MS/MS and immunoassay methods (R^2=0.99).

CONCLUSIONS: CRP in plasma can be quantified by HPLC-MS/MS with preliminary data indicating performance comparable to an existing clinical immunoassay. Critically, the new method requires significantly less sample volume compared to routine clinical methods.

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GRANZYME K IMPAIRS BURN WOUND HEALING

Christopher Turner[1], Hongyan Zhao[1], Philip Bird[2], Anthony Papp[3], David Granville[1]

BACKGROUND/OBJECTIVES: Burns are the most extensive form of soft tissue injury, often leading to excessive scarring and skin contractures. Treatment of burns is a complex medical problem with multiple methods applied to treat such injuries, including dressings, antimicrobials and use of skin substitutes. The healing of burn wounds requires an intricate coordination of events requiring interaction between multiple cell types and the extracellular microenvironment. Wound healing can be divided into four stages: hemostasis, inflammation, proliferation and tissue remodeling. Curbing excessive inflammation is associated with improved wound healing outcomes.

Granzyme K (GzmK) is a serine protease found at low levels in the plasma of healthy individuals, but is markedly elevated during inflammatory events, including viral infections and sepsis. GzmK promotes inflammation, triggering the increased release of pro-inflammatory markers, namely interleukin-6 (IL-6) and monocyte chemoattractant protein -1 (MCP-1), in lung fibroblasts and endothelial cells, with this functioning through a protease-activated receptor-1 (PAR-1) mediated pathway. Elevated GzmK therefore corresponds to increased disease severity in inflammatory disease. It is hypothesized that GzmK is increased in burn injury, and that elevated GzmK contributes to prolonged inflammation, impaired wound healing, fibrosis and scarring.

RESULTS: GzmK expression was evaluated in human burn wounds, showing elevated GzmK in all burns analyzed compared to healthy skin. A 6-day burn trial was performed in wild-type mice, involving a single burn on the dorsum of 8 week old female mice. Immunohistochemistry showed a small but significant increase in GzmK in the dermis at the wound margin by d3, with staining intensity greatly increased by d6. An additional burn trial was performed, this time involving a GzmK knockout (KO) mouse model. Macroscopically, there was a significant reduction in wound area from d5 compared to equivalent burns in wild-type mice. This improvement corresponded to wound closure approximately one day earlier than wild-type mice. Cell culture studies were performed to further investigate the role of GzmK in impaired cutaneous wound repair. Here, recombinant human GzmK (rhGzmK) induced IL-6 release from both human skin fibroblasts and keratinocytes (HaCaTs). GzmK altered cytokine expression in a dose-dependent manner, and with no induction of cytotoxicity. To our knowledge, this provides the first report of GzmK release from keratinocytes. The effect of GzmK on the recovery of cultured keratinocytes in response to wounding was evaluated using Electric Cell-substrate Impedance Sensing (ECIS). Keratinocytes treated with rhGzmK 3 h prior to wounding showed improved wound repair compared to vehicle-treated controls.

CONCLUSIONS: The burn area in GzmK KO mice was significantly reduced compared to equivalent burns in wild-type controls, suggesting GzmK contributes to impaired healing. GzmK induces IL-6 release from epithelial cells, indicating the pro-inflammatory stage of wound healing may be prolonged.

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THE MOLECULAR DIFFERENCE BETWEEN CENTRILOBULAR AND PANLOBULAR EMPHYSEMA

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BACKGROUND/OBJECTIVES: Centrilobular (CLE) and panlobular (PLE) emphysema are major pathological phenotypes of COPD. However, little is known about their molecular differences. The purpose of this study is to compare the molecular signatures of CLE and PLE.

METHODS: MicroCT, quantitative histology for immune cells, and gene expression profiling were performed on 40 samples from 5 PLE, 5 CLE, and 5 control lungs respectively. Linear-mixed-effect model (LMM), immune-network-analysis and Non-negative-matrix-factorization (NMF) were used to evaluate the differences between CLE and PLE.

RESULTS: Gene lists established by LMM which were associated with emphysema severity measured by mean linear intercept (Lm) or the infiltrating cells had small overlap between CLE and PLE subjects. Low similarity of subsequent function and pathway enrichment analysis results further confirm the differences. The immune-network-analysis showed almost no direct connections between Lm related genes in CLE and PLE. Further, different expression patterns between CLE and PLE samples were discovered by NMF analysis.

CONCLUSIONS: The combination of LMM, network analysis, and NMF reveal significant differences in subjects with PLE and CLE at the molecular level. The mechanisms of these differences will be clarified in subsequent studies.

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MASS SPECTROMETRIC IDENTIFICATION OF THE PLASMA ACTH-OME

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BACKGROUND/OBJECTIVES: Adrenocorticotropic hormone (ACTH) is a 39-residue peptide hormone that stimulates cortisol release from the adrenal gland. Residues 1-24 of ACTH are sufficient for normal biological activity. As there is evidence that pituitary tissue contain an array of proteolytic fragments of ACTH, we were interested in determining whether these fragments were found circulating in significant quantities in blood (i.e. characterizing the plasma ACTH-ome). Moreover, as sandwich immunoassays are commonly employed to quantitate plasma ACTH, we were interested in the potential for these fragments to interfere with clinical testing.

METHODS: Analyses included immunoprecipitation (IP) of ACTH peptides from plasma or stock solutions followed by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) or liquid chromatography (LC)-tandem MS. For the IP step, we used the capture antibodies (Ab) from two commercially available clinical immunoassay kits: Roche Elecsys® ACTH and Siemens Immulite® ACTH 2000. Ab saturation curve were built for each IP protocol using serial diluted synthetic mouse ACTH. As the epitopes of the antibodies were not publically available, we first epitope mapped the antibodies via IP of various synthetic ACTH peptides fragments followed by MALDI-TOF MS. For investigation of the plasma ACTH-ome, we selected fragments previously identified in pituitary tissue (7-31, 7-34, 7-36, 7-38, 7-39, 1-31, 1-37 and 1-38), as well as, used our IP-MALDI method to identify abundant ACTH fragments in plasma. These ACTH fragments were then used to develop a targeted multiple reaction monitoring (MRM) IP-LC-MS/MS method. Ongoing analyses include using the MRM method to characterize the plasma ACTH-ome of healthy controls and individuals with disorders of the hypothalamic–pituitary–adrenal axis.

RESULTS: Epitope mapping studies revealed that the capture and detection Ab of the Elecsys® ACTH kit were directed against residues 9-12 and 36-39, respectively. The IP methods were linear up to ACTH concentrations of at least 290 pmol/L (R2 > 0.99) [Elecsys® capture Ab] and 318 pmol/L (R^2 > 0.99) [Immulite® capture Ab]. The detection limit of the IP-LC-MS/MS method was 2 pmol/L of ACTH, signal/noise > 15. Multiple ACTH fragments were identified in plasma by MALDI-TOF MS and subsequently subject to MRM development including: 1-39, 7-38, 1-38, 7-35, 5-39 and 8-26. We also developed MRMs for 1-24, 18-39 and mouse ACTH 1-39.

CONCLUSIONS: In this study, we have identified multiple ACTH fragments in human plasma in addition to full-length ACTH using non-targeted and targeted MS. Interestingly, many of the fragments in the plasma ACTH-ome lacked a portion of the N-terminus but maintained the full epitopes required for reactivity with the immunoassay. Such N-terminal truncations of ACTH are known to result in reduced biological activity, and in certain cases act as ACTH-receptor antagonists. Therefore, routinely used clinical immunoassays are at risk for interference from biologically inactive but assay-reactive ACTH species, requiring cautious interpretation of plasma ACTH results.

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PROPHYLACTIC ANTIGEN MATCHED DONOR BLOOD FOR PATIENTS WITH WARM REACTIVE IGG AUTOANTIBODIES: AN EVALUATION OF SAFETY AND EFFICACY

Hamish Nicolson[1], Lawrence Sham[2], Kristine Roland[3]

BACKGROUND/OBJECTIVES: We performed a quality assurance review to evaluate the safety and efficacy of prophylactic antigen-matched donor blood for patients with warm-reactive IgG autoantibodies (WAA) over the last seven years at Vancouver General Hospital (VGH).

METHODS: This was a retrospective review of all patients with a WAA detected by our transfusion medicine service from January 1, 2008 to Jan 1, 2016. The electronic patient record was used to identify all patients with a WAA designation and their corresponding diagnoses, laboratory parameters (reticulocytosis, indirect bilirubin, LDH), red blood cell (RBC) and platelet (PLT) transfusions, alloantibodies and timing of alloantibody formation

RESULTS: A total of 480 patients were designated as having WAA during the study period. Thirty patients (6%) designated as having WAA during the study period developed new alloantibodies after that designation was made. The average age of these patients was 73 (range 37 - 96). These patients included 17 females and 13 males. The majority of these patients (73%) had history of either autoimmune, chronic inflammatory, benign or malignant hematological disease (Fig 1). Despite our policy of issuing Rh and Kell-matched RBCs, 29 of the 50 (58%) new alloantibodies formed were Rh or Kell (Table 1). Of these 29 patients, 6 had a known history of autoimmune hemolytic anemia and 4 of the 6 had laboratory evidence of hemolysis. Of the remaining 21 alloantibodies formed, half (10) were Kidd, and the others made up by a variety of blood group systems (Table 1). Nine of the 30 patients received PLT transfusions.

CONCLUSIONS: A policy of providing prophylactically matched RBCs to patients with active WAA does not completely prevent the formation of new alloantibodies. Despite the formation of these new alloantibodies, the incidence of hemolysis in the setting of WAA is low. Prophylactically matching for Kidd (in addition to Rh and Kell) may be indicated.

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WILD WATERFOWL-INDEPENDENT AVIAN INFLUENZA VIRUS SURVEILLANCE AND CHARACTERIZATION USING REAL-TIME PCR AND ENRICHMENT-BASED TARGETED RESEQUENCING OF WETLAND SEDIMENTS

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BACKGROUND/OBJECTIVES: Avian influenza is an economically important disease that can decimate the poultry industry and is a threat to public health. Early virus detection and characterization is the key to successful avian influenza surveillance. However, the current dead-bird surveillance system in British Columbia failed to predict the incursion of avian influenza virus (AIV) during the 2014/15 season. We therefore explored the applicability of a molecular strategy for virus detection in wetland sediments contaminated with bird feces during seasonal migration as a population level proxy of AIV activity in waterfowls. The wetlands can potentially capture the diversity of influenza A viruses from multiple avian host and therefore act as sentinel samples that can be used for high-throughput active surveillance of AIV of wild migratory birds.

METHODS: A total of 300 sediment samples from 15 wetland areas in Fraser Valley of British Columbia ranging from <1- 280 hectares were selected, based on their proximity to AIV infected poultry farms and the diversity and abundance of migrating birds in the area using a citizen-science bird database (www.ebird.org). Total RNA from wetland sediment samples was extracted using an optimized protocol and screened for AIV using matrix gene real time RT-PCR (qRT-PCR). Prior to next-generation sequencing, target enrichment for AIV matrix (M), hemagglutinin (HA) and neuraminadase (NA) genes of the RNA extracts was performed using PCR and liquid-phase hybridization capture-based approaches. The H5, N2 and N1 with longer consensus sequences were included in the phylogenetic analysis using maximum-likelihood phylogeny.

RESULTS: The qRT-PCR analysis revealed that around eight percent (23/300) of the sediment samples were positive for influenza A virus matrix gene. Target capture resequencing identified the M gene and at least one HA and NA subtype in 28.7% (86/300) of the wetland samples. A maximum number of five HA and five NA subtypes were detected in one sample. A total of 11 HA (H1, H3-7, H9-12, H16) and seven NA (N1-3, N5, N7-9) subtypes across different wetland sites were identified and characterized. The commonly identified subtypes in the samples include H10 (6.3%), H11 (6.3%) and H5 (6%) for HA and N2 (7.3%), N7 (5.7%), and N9 (6%) for NA. Phylogenic analysis revealed that the outbreak-associated HPAI H5 sequences from 5 (1.7%) RNA extracts formed a distinct clade with Eurasian-origin H5 HPAI outbreak clade 2.3.4.4 and the 2014/2015 BC poultry H5 pandemic sequences. In addition, the data showed that although greater diversity exist in N2 and N1 sequences, most of the samples grouped within North American clades. Furthermore, majority of N2 sequences clustered with the H5N2 isolates from domestic and wild birds in the USA and Canada.

CONCLUSIONS: The matrix gene qRT-PCR is a useful screening assay for AIV detection in sediment samples. The targeted resequencing method was more sensitive and provided subtyping characterization. The study reports on a new laboratory culture-independent method for AIV detection and sustainable wild bird sample-independent AIV surveillance system. The alternative AIV surveillance strategy can be used with the existing wild bird surveillance programs to provide an early warning plan for AIV poultry outbreaks or potential AIV pandemic threats in Canada and worldwide.

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GENERATION OF PAEDIATRIC REFERENCE INTERVALS FOR BLOOD DETECTION OF THE BRAIN PROTEIN TAU

Sophie Stukas[1], Victoria Higgins[2], Khosrow Adeli[2], Cheryl Wellington[1]

BACKGROUND/OBJECTIVES: Blood-based biomarkers to assist in the diagnosis and prognosis of TBI are highly desirable. This is especially true for paediatric cases, as infants cannot speak for themselves and neuroimaging can be complex. Another critical gap is the paucity of uninjured controls against which biomarker changes are evaluated. Reference intervals (RI) enable clinicians to confidently interpret test results as normal or abnormal and are critical for accurate diagnosis and for prognostic judgment. Paediatric RI can be difficult to establish because multiple age and sex partitions are often required to reflect the rapid changes that occur during growth and development. Challenges associated with recruiting newborns and children, coupled with the small blood volumes that can be collected, limit sample size and subsequent robust calculations from a representative population. Many past studies have relied on outdated technology, used retrospective laboratory data or collected samples from hospitalized patients rather than healthy children. Therefore, a complete, accurate, and up-to-date database of paediatric reference intervals for clinically relevant biomarkers is urgently needed, especially for TBI.

HYPOTHESIS AND OBJECTIVES: In adults, serum and plasma levels of the neuronal protein tau rise rapidly post TBI, correlating with the severity and resolution of post concussion symptoms and return to play. We hypothesize that serum tau will be elevated following TBI in paediatric subjects and that it may correlate with prognostic outcome. The specific objective of this study is to generate paediatric RI for serum tau to allow for future comparison of tau measured in paediatric TBI patients.

METHODS: 309 control paediatric serum samples from males and females age 1-18 were drawn from CALIPER (Canadian Laboratory Initiative on Paediatric Reference Intervals) and serum tau was measured using the Simoa HD-1 analyzer (Quanterix), a fully automated digital ELISA platform, using commercially available immunoassays. The Harris and Boyd statistical method was used to determine statistically relevant age and sex partitions and the normality of each partition was testing using the Shapiro-Wilk test and Q-Q plots. Outliers were removed using the Tukey test twice, for normally distributed data, or adjusted Tukey test twice, for skewed data. RI were calculated using the nonparametric rank method and 90% confidence intervals around each reference limit were calculated using ranked observations.

RESULTS AND SIGNIFICANCE: While there was no correlation with sex, there was a significant negative correlation between age and serum tau, with the highest levels observed in children under 4. Statistical analysis revealed 4 age partitions with lower and upper RI values for tau as follows: 0- <4 years (N=48, 0.83-20.15 pg/ml); 4- <9 years (N=56, 1.41-5.10 pg/ml); 9-<15 years (N=118, 0.83-4.92 pg/ml); and 15- <19 years (N=75, 1.00-4.70). Serum tau, therefore, may be a strong diagnostic blood-based biomarker following TBI in paediatric patients between ages 4- <19 years given the tight data distribution and homogeneity.

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