PATHOLOGY
laboratory medicine
FACULTY OF MEDICINE
UNIVERSITY OF BRITISH COLUMBIA

Pathology Day is a critically significant event in the departmental calendar as it serves as a time to showcase scholarly activities, including basic investigative, translational, and clinical-applied research, performed by our trainees and, by extension, our faculty.

ABSTRACT BOOK
PATHOLOGY DAY 2021

Follow us on Twitter @ ubcpathology / tag your tweets with #UBCPathDay2021

www.pathology.ubc.ca

KEYNOTE SPEAKER

SRIRAM SUBRAMANIAM, PHD

Gobind Khorana Canada Excellence Research Chair (CERC) in Precision Cancer Drug Design; Professor, Department of Biochemistry and Molecular Biology

TITLE: “Visualizing biological complexity in 3D”

For this years Pathology Day event, we are excited to be using the virtual platform Gather town https://gather.town. The Pathology Day virtual space on Gather.town will be similar to an ‘onsite’ conference, where you can meet your colleagues, friends, and other attendees to strike up conversations just like you would at an in-person event. Further, it allows attendees to view talks by visiting specific rooms, and an interactive poster session that facilitates walking through a room, visiting those of interest and asking questions directly to the presenter face to face.

JAMES HOGG LECTURER

POUL SORENSEN, MD, PHD

TITLE: "Stress management in the tumor cell workplace--lessons learned from studying high-risk pediatric sarcomas"

GUEST SPEAKER

RAMON KLEIN GELTINK, PHD

TITLE: "Immunometabolism for breakfast: are you always what you eat?"

GUEST SPEAKER

LISA OSBORNE, PHD

TITLE: "Origin stories: effects of intestinal insults on peripheral immunity and inflammation"

SELECTED PRESENTATION

MICHAEL LIZARDO, STAFF SCIENTIST

TITLE: "The rocaglate eukaryotic initiation factor 4A-1/2 inhibitor CR-1-31B has both anti-tumour and anti-metastatic activity in osteosarcoma"

PATHOLOGY DAY 2021
MAY 28, 2021

PATHOLOGY laboratory medicine
FACULTY OF MEDICINE
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CONFERENCE OUTLINE

7:55 am – 8:05 am OPENING REMARKS – **DR. DON BROOKS**	https://ubc.zoom.us/j/64457262783?pwd=Wm5UOXAvSHB3dkkvcElLSytyME12UT09 [room: key note; pwd: 074793; mtg id: 644 5726 2783]

+ ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS [room: concurrent A pwd: 740147, mtg id: 655 4009 0154]		**RESIDENTS** [room: concurrent B pwd: 478724, mtg id: 675 6941 8114]	
https://ubc.zoom.us/j/65540090154?pwd=Q2RnZEpEN1c4T2Q2YnZIU-FRENnVFZz09		https://ubc.zoom.us/j/67569418114?pwd=N1dpSWE3UjdaKzRxdjBCSH-JtRDhmZz09	
8:10 am – 8:25 am	**Sofya Langman**	**8:10 am – 8:25 am**	**Hang Yang**
8:25 am – 8:40 am	**Katlyn Richardson**	**8:25 am – 8:40 am**	**Julia Naso**
8:40 am – 8:55 am	**Emel Islamzada**	**8:40 am – 8:55 am**	**Hezhen Ren**

9:00 am – 9:20 am GUEST SPEAKER: **DR. RAMON KLEIN GELTINK**	**TITLE: *"Immunometabolism for breakfast: are you always what you eat?"*** https://ubc.zoom.us/j/61434460903?pwd=U05HVlRLdkV4Y0daOG5Xczh3SERmdz09 [room: key note; pwd: 634345; mtg id: 614 3446 0903]
9:20 am – 9:50 am TRIBUTE - DR. **DAVID HARDWICK**	https://ubc.zoom.us/j/61434460903?pwd=U05HVlRLdkV4Y0daOG5Xczh3SERmdz09 [room: key note; pwd: 634345; mtg id: 614 3446 0903]
9:50 am – 10:05 am	**BREAK**
10:05 am – 11:05 am JAMES HOGG LECTURE: **DR. POUL SORENSEN**	**TITLE: *"Stress management in the tumor cell workplace--lessons learned from studying high-risk pediatric sarcomas"*** https://ubc.zoom.us/j/67833439579?pwd=VVlTaTBOZ3lvdGtLUm5rMCtOMjN4UT09 [room: key note; pwd: 886050; mtg id: 678 3343 9579]

+ ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS room concurrent A		**RESIDENTS** room concurrent B		**RESIDENTS** room concurrent C	
pwd: 278313, mtg id: 652 2783 8898 https://ubc.zoom.us/j/65227838898?pwd=cE-1mR2s4clY1bUVjUnpBbHdiekYyQT09		pwd: 610768, mtg id: 663 0734 4033 https://ubc.zoom.us/j/66307344033?pwd=O-FoxbmM1bUI2ZFY2QVlicjhiUTNJdz09		pwd: 938066, mtg id: 693 9748 3154 https://ubc.zoom.us/j/69397483154?pwd=Mn-RneW5CT1dST1lReTNldDJFOXJqQT09	
11:15 am – 11:30 am	**Jennifer Ji**	**11:15 am – 11:30 am**	**Jonathan Lee**	**11:15 am – 11:30 am**	**Sam Chorlton**
11:30 am – 11:45 am	**Elyn Rowe**	**11:30 am – 11:45 am**	**Yi Ariel Liu**	**11:30 am – 11:45 am**	**Michael Multan**
11:45 am – 12:00 pm	**Kevin Kuchinski**	**11:45 am – 12:00 pm**	**Bradley Chaharyn**	**11:45 am – 12:00 pm**	**Ann Tran**

12:10 pm – 2:10 pm	(VIRTUAL ON GATHER TOWN - instructions on how to join through Gather town)
2:20 pm – 2:40 pm GUEST SPEAKER: **DR. LISA OSBORNE**	**TITLE: *"Origin stories: effects of intestinal insults on peripheral immunity and inflammation"*** https://ubc.zoom.us/j/62707058038?pwd=b2EyQzNuSDR3ZFNYUGdob2o1ZFFUZz09 [room: key note; pwd: 019235; mtg id: 627 0705 8038]

+ ORAL PRESENTATIONS BY STUDENTS & RESIDENTS

GRAD STUDENTS room Concurrent A		**RESIDENTS** room Concurrent B		**RESIDENTS** room Concurrent C	
pwd: 374562, mtg id: 625 4857 8062 https://ubc.zoom.us/j/62548578062?pwd=TFV-4VmY3UDZicnJCbUkvOWZjQzJTZz09		pwd: 228386, mtg id: 695 9298 2508 https://ubc.zoom.us/j/69592982508?pwd=OD-JVSVdzL3orZW1JRWxmQytiMmE4UT09		pwd: 882266, mtg id: 611 3009 0045 https://ubc.zoom.us/j/61130090045?pwd=R-ko1RkxxUks2SjdDNlpWQWNmT0I2Zz09	
2:50 pm – 3:05 pm	**Brett Collinge**	**2:50 pm – 3:05 pm**	**Michael Steel**	**2:50 pm – 3:05 pm**	**Taylor Salisbury**
3:05 pm – 3:20 pm	**Han Qi Zhao**	**3:05 pm – 3:20 pm**	**Jenny Chu**	**3:05 pm – 3:20 pm**	**Hussam Bukhari**
3:20 pm – 3:35 pm	**Rachel Cederberg**	**3:20 pm – 3:35 pm**	**Lisa Borretta**	**3:20 pm – 3:35 pm**	**Kimberly Hamilton**

3:55 pm – 4:15 pm STAFF GUEST SPEAKER: **DR. MICHAEL LIZARDO**	**TITLE: *"The rocaglate eukaryotic initiation factor 4A-1/2 inhibitor CR-1-31B has both anti-tumour and anti-metastatic activity in osteosarcoma"*** https://ubc.zoom.us/j/62634407038?pwd=SWgzdVY4VXkrK1F1V3drbk41Q0R2QT09 [room: key note; pwd: 050948; mtg id: 626 3440 7038]
4:15 pm – 4:30 pm	**BREAK**
4:30 pm – 5:30 pm KEYNOTE SPEAKER: **DR. SRIRAM SUBRAMANIAM**	**TITLE: "Visualizing biological vomplexity in 3D"** https://ubc.zoom.us/j/69058234502?pwd=UXMrNFpzbEhIZTBtOG9PeDdJZTE2Zz09 [room: key note; pwd: 054477; mtg id: 690 5823 4502]
5:45 pm – 6:00 pm	**BREAK**
6:00 pm – 8:30 pm	**AWARDS** https://ubc.zoom.us/j/66527273347?pwd=Z0ZkK2VweElEMUs4QUpMRHp5SU12QT09 [room: key note; pwd: 313724, mtg id: 665 2727 3347]

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Zeshuo Li[1,2], Rachel Dunn[1,2], Hélène CF Côté[1,2], and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (Hélène CF Côté CARMA-PREG)

48 FANG FANG LI [graduate student]

ASSAYS TO MEASURE THE IMPACT OF PREVIOUS CORONAVIRUS INFECTION ON THE IMMUNE RESPONSE GENERATED FROM SARS-COV-2 INFECTION AND VACCINATION

Fang Fang Li[1], Aaron Liu[2], Ebrima Gibbs[3], Guadalein Tanunliong[4], Jun Duan[1,4], Natalie Prystajecky[1,4], Mel Krajden[1,4], Muhammad Morshed[1,4], Soren Gantt[5], Neil Cashman[3], Inna Sekirov[1,4], Agatha Jassem[1,4]

49 MICHAEL LI [graduate student]

TRAF3 LOSS-OF-FUNCTION DRIVES NON-CANONICAL NF-KB PATHWAY ACTIVATION IN DIFFUSE LARGE B-CELL LYMPHOMA

Michael Y Li[1,2], Lauren C. Chong[1], Elizabeth A Chavez[1], Bruce W Woolcock[1], Adele Telenius[1], Vivian Lam[3], Gerald Krystal[3], Elena Vigano[1], Shannon Healy[1], David W. Scott[1,2] and Christian Steidl[1,2]

50 JENNIFER LUU [graduate student]

IDENTIFYING SECRETED PROTEINS DURING MALIGNANT TRANSFORMATION OF LUNG EPITHELIAL CELLS

Jennifer Luu[1,2], Fraser Johnson[2,3], Will Lockwood[1,2]

51 RANA MINAB [graduate student]

BRAIN LIPIDS ARE RECOGNIZED BY ANTIBODIES MADE DURING INFECTIOUS MONONUCLEOSIS – ITS IMPLICATIONS FOR MULTIPLE SCLEROSIS AND OTHER AUTOIMMUNE NEUROLOGICAL DISEASES

Rana Minab[1], Sharad Purohit[2], Dong Zheng Jun[3], Mairead Cavinaw[3], Peter van den Elzen[1]

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52 ANGELA MO [graduate student]

ELUCIDATING THE MECHANISMS OF LEUKEMOGENESIS DRIVEN BY FBXO11 LOSS

Angela Mo[1,2], Linda Chang[1,2], Gerben Duns[1], Xuan Wang[2], Elijah Willie[2], Shujun Huang[2], Derek Tam[2], Nadia Gharaee[2], Rawa Ibrahim[1,2], Rod Docking[1,2], Patricia Umlandt[1,2], Jeremy Parker[1,2], Aly Karsan[1,2]

53 KOUTHER NOUREDDINE [graduate student]

COMBINING MULTIPLEXED IMMUNO-HISTOCHEMISTRY AND DEEP LEARNING TO SPATIALLY MAP THE TUMOUR MICROENVIRONMENT

Kouther Noureddine[1], Paul Gallagher[1], Martial Guillaud[1,3], Calum MacAulay[1,2]

54 JUHEE OH [graduate student]

NUTRIENT STRESS-INDUCED KINASE SIGNALING ENHANCES PRIMARY CD8+ T CELL FUNCTION

Juhee Oh[1,2], Annette E Patterson[1,2], Neeku Amanat[1,2], Janice Tsui[1,2], Anuli C Uzozie[1,2], Kirsten A Ward-Hartstonge[1,3], Philipp F Lange[1,2], and Ramon I Klein Geltink[1,2*]

55 MANIDEEP CHOWDARY PACHVA [graduate student]

DETERMINING THE ROLE OF EWING SARCOMA DERIVED EXTRACELLULAR VESICLES IN REGULATING THE TUMOR MICROENVIRONMENT

Manideep Chowdary Pachva[1]

56 LINDSAY PALLO [graduate student]

DEVELOPMENT OF AN ASSAY FOR (PRO)-ISLET AMYLOID POLYPEPTIDE (1-67) IMMUNOREACTIVITY

Lindsay Pallo[1,2], Yi-Chun Chen[1,3], Jaques Courtade[1,2], Bruce Verchere[2,3]

57 MEGAN PAWLUK [graduate student]

GRANZYME B: A NOVEL THERAPEUTIC TARGET FOR RADIATION DERMATITIS

Pawluk, Megan A[1,2], Hiroyasu, Sho[1,2], Nabai, Layla[1,2], Wadsworth, Brennan. J[2,3], Shen, Yue[1,2], Bennewith, Kevin L[2,3], Granville, David J[1,2,4]

58 TETIANA POVSHEDNA [graduate student]

RISK SCORES FOR AGE-RELATED COMORBIDITIES AND IMMUNE AGING MARKERS IN WOMEN OVER 45 YEARS OLD LIVING WITH OR WITHOUT HIV IN THE CARMA COHORT IN BRITISH COLUMBIA: A PRELIMINARY ANALYSIS

Tetiana Povshedna[1], Maya Rosenkrantz[2], Anthony YY Hsieh[1], Jonathan Steif[3], Arianne Albert[4,5,6], Evelyn J Maan[5], Beheroze Sattha1,5, Ariel Nesbitt[2,5], Shanlea Gordon[4], Jerilynn Prior[2,4,7], Deborah M. Money[4,5,6], Melanie CM Murray[4,5,8], Neora Pick[4,5,8], Hélène C.F. Côté[1,4], for the CIHR Team Grant on Cellular Aging and HIV Comorbidities in Women and Children and the CARMA (Children and Women: AntiRetrovirals and Markers of Aging) cohort

59 FARNAZ SAHRAGARD [graduate student]

TESTING THE SAFETY OF AN OPTICAL SENSOR DESIGNED TO MONITOR SPINAL CORD HEMODYNAMICS

Farnaz Sahragard[1,2], Amanda Cheung[1], Femke Streijger[1], Shahbaz Askari[1,3], Brian kwon[1,3,4], Babak Shadgan[1,2,3,4]

60 CHAE YOUNG SHIN [graduate student]

INVESTIGATING THE TUMORIGENIC ROLE OF ARID1B LOSS IN ARID1A-MUTATED DEDIFFERENTIATED ENDOMETRIAL CARCINOMA

Chae Young Shin[1,2], Raymond Zirui Feng[2], Valerie Lan Tao[2], Maxwell Douglas[2], Shelby Thornton[2], Lien Hoang[3], Yemin Wang[1,2], David Huntsman[1,2]

61 TARAS SHYP [graduate student]

SIX-TRANSMEMBRANE EPITHELIAL ANTIGEN OF THE PROSTATE 1 FACILITATES IRON TRANSPORT IN EWING SARCOMA TO SUPPORT MITOCHONDRIAL ACTIVITY

Taras Shyp[1,2], Rouhollah Mousavizadeh[1], Gian Luca Negri[3], Alberto Delaidelli[1,2], Andrii Vislovukh[1,2], Hai-Feng Zhang[2], Michael Lizardo[2], Poul Sorensen[1,2]

62 TIANNA SIHOTA [graduate student]

CHARACTERIZATION OF A NOVEL TUMOR SUPPRESSOR AND RISK FACTOR IN LUNG ADENOCARCINOMA

Tianna S Sihota[1,2], Amy Nagelberg[1,2], Rocky Shi[2,3], Fraser D Johnson[2,3], and William W Lockwood[1,2,3]

63 MARIE-SOLEIL SMITH [graduate student]

ROBUST TOBACCO SMOKING SELF-REPORT IN TWO PAN-CANADIAN COHORTS: PREGNANT WOMEN OR MEN AND WOMEN LIVING WITH OR WITHOUT HIV

Marie-Soleil R. Smith[1,2], Sara Saberi[1,2], Abhinav Ajaykumar[1,2], Mayanne MT Zhu[1], Izabelle Gadawski[1,2], Beheroze Sattha[1], Evelyn J Maan[3,4], Julie Van Schalkwyk[3,4,5], Chelsea Elwood[3,5], Neora Pick[3,5,6], Melanie C.M. Murray[3,5,6], Isabelle Boucoiran[7], Deborah M. Money[3,4,5], Hélène C.F. Côté[1,2,3], and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

64 DARCY SUTHERLAND [graduate student]

SYNTHETIC ANTIMICROBIAL PEPTIDES KILL MULTI-DRUG RESISTANT PATHOGENS IN VITRO

Darcy Sutherland[1,2,3], S Austin Hammond[1], Chenkai Li[1,4], René L Warren[1], Caren Helbing[5], Titus Wong[5], Linda Hoang[2,3], Mel Krajden[2,3] and İnanç Birol[1,2,3,7]

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65 BUSRA TURGU [graduate student]

HACE1 BLOCKS HIF1A ACCUMULATION UNDER HYPOXIA IN A RAC1 DEPENDENT MANNER

Busra Turgu[1,2], Fan Zhang[3], Amal El-Naggar[1,4,5], Gian Luca Negri[6], Melanie Kogler[7], Luigi Tortola[7,8], Fraser Johnson[1], Tony Ng[4], Amy Li[1], Donald Yapp[9], William Lockwood[4,10], Daniel Martinez[11], John M Maris[12], Mads Daugaard[3], Josef M Penninger[7,13], Christopher S Hughes[1], Poul H Sorensen[14,15]

66 HENRY WEST [graduate student]

THE ROLE OF TISSUE FACTOR IN VIRAL INFECTION AND PATHOLOGY

Henry West[1,3], Alex Leatherdale[1,3], Bryan Lin[1,3], Michael Sutherland[1,3,4], Ed Conway[1,2,3], Ed Pryzdial[1,3,4]

67 NANCY YANG [graduate student]

THE IMPACT OF CHRONIC AND LATENT VIRAL INFECTIONS ON AGING IN PEOPLE LIVING WITH HIV

Nancy Yang[1,2], Zhuo Chen[1], Hélène CF Côte[1,2], for the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA cohort)

68 JOYCE ZHANG [graduate student]

LOW-GRADE SEROUS OVARIAN CANCERS MODELLED WITH HUMAN FALLOPIAN TUBE ORGANOIDS AND SINGLE CELL SEQUENCING

Joyce Zhang[1,2], Dawn Cochrane[1], Kieran Campbell[1], Minh Bui[1], Germain Ho[1], Genny Trigo[1], Winnie Yang[1], Cindy Shen[1], Sorab Shah[1], David Huntsman[1,2]

69 GUANGZE ZHAO [graduate student]

KNOCKDOWN OF NUCLEAR FACTOR OF ACTIVATED T-CELLS 5 PROMOTES THE COXSACKIEVIRUS B3-INDUCED MYOCARDITIS

Guangze Zhao[1,2], Mary Huifang Zhang[1,2], Fione Yip[1,2], Sana Aghakeshmiri[1,2], Terry Chen[1,2,] Christoph Kuper[3], Decheng Yang*[1,2]

70 TOM CHENG [postdoctoral fellow]

CHIMERA TRAUMATIC BRAIN INJURY IN A MOUSE MODEL OF TAUOPATHY

Wai Hang Cheng[1], Grace Hu[1], Jianjia Fan[1], Honor Cheung[1], Jennifer Cooper[1], Carlos J Barron[1], Peter A Cripton[2], David Vocadlo[3], Cheryl L Wellington[1]

71 MONA KHORSHIDFAR [postdoctoral fellow]

QUALITY MEASUREMENTS OF APHERESIS PLATELET CONCENTRATES FROM DONORS WHOSE PLATELETS REPEATEDLY SHOW LOW PH OVER STORAGE

Mona Khorshidfar[1], Katherine Serrano[1,2], Mary Huang[3], Dana V. Devine[1,2]

72 HAI-FENG ZHANG [postdoctoral fellow]

PROTEOMIC SCREENS FOR SUPPRESSORS OF ANOIKIS IDENTIFY IL1RAP AS A NEW SURFACE TARGET FOR IMMUNOTHERAPY IN EWING SARCOMA

Hai-Feng Zhang[1,2], Christopher S Hughes[1,2], Wei Li[3], Jianzhong He[4], Didier Surdez[5], Amal M El-Naggar[1,2], Hongwei Cheng[2], Anna Prudova[2,6], Alberto Delaidelli[1,2], Gian Luca Negri[6], Xiaojun Li[2], Maj S Ørum-Madsen[7], Michael M Lizardo[2], Htoo Zarni Oo[7], Shane Colborne[6], Taras Shyp[1,2], Renata Scopim Ribeiro[1,2], Colin A Hammond[8], Anne-Chloe Dhez[1,2], Sofya Langman[1,2], Jonathan KM Lim[1,2], Sonia HY Kung[7], Amy Li[1,2], Anne Steino[2], Mads Daugaard[7], Rimas J Orentas[9], Li-Yan Xu[4], Gregg B Morin[6,10], Olivier Delattre[5], Dimiter S Dimitrov[3], Poul H Sorensen[1,2]

73 NARGES HADJESFANDIARI [research associate]

FATTY WHOLE BLOOD DONATIONS AND HIGH RED BLOOD CELL STORAGE HEMOLYSIS

Narges Hadjesfandiari[1,2], Katherine Serrano[1,2], Elena Levin[1,2], Caesar DiFranco[2] and Dana Devine[1,2]

74 KATHERINE SERRANO [research associate]

RISK ANALYSIS OF TRANSFUSION OF CRYOPRECIPITATE WITHOUT CONSIDERATION OF ABO GROUP

Katherine Serrano[1,2], Narges Hadjesfandiari[1,2], Elena Levin[1,2], Qi-Long Yi[2], Dana Devine[1,2]

75 RYAN PENNY [staff]

EVALUATION OF THE NG-TEST CARBA 5 MULTIPLEX IMMUNOCHROMATOGRAPHIC ASSAY AND CEPHEID XPERT CARBA-R ASSAY FOR THE DETECTION OF CARBAPENEMASE GENES IN GRAM NEGATIVE ORGANISMS

Ghada N Al-Rawahi[1,2], Ryan Penny[1], Audrey Cuarte[1], Suk Dhaliwal[1], Tammy Chen[1], Dale Purych[3,2], David Boyd[4], Linda Hoang[5,2], Peter Tilley[1,2]

76 MICHAEL LIZARDO [staff - SELECTED FOR ORAL PRESENTATION]

THE ROCAGLATE EUKARYOTIC INITIATION FACTOR 4A-1/2 INHIBITOR CR-1-31B HAS BOTH ANTI-TUMOUR AND ANTI-METASTATIC ACTIVITY IN OSTEOSARCOMA

Michael M Lizardo[1] , Yue Zhou Huang[1], Christopher Hughes[1], Sofya Langman[1], and Poul H Sorensen[1,2]

Sofya Langman

GRADUATE STUDENT

ABSTRACT #1

Supervisor: Dr. Poul Sorensen

Sofya Langman[1,4]; Sean W. Minaker[2]; Christopher S. Hughes[1]; Fiona Zhang[3]; Alberto Delaidelli[1]; Yuzhuo Wang[3]; Poul H. Sorensen[1]

Affiliations:
[1]Department of Molecular Oncology, British Columbia Cancer Research Centre; [2]Terry Fox Laboratory, British Columbia Cancer Research Centre; [3]Department of Experimental Therapeutics, British Columbia Cancer Research Centre; [4]Interdisciplinary Oncology Program, University of British Columbia

UBIQUITIN-ASSOCIATED-PROTEIN-2-LIKE PROTEIN (UBAP2L) FACILITATES EWING SARCOMA METASTASIS BY PROTECTING CORE STRESS GRANULE NUCLEATOR FROM PROTEASOMAL DEGRADATION

Background/objectives: Ewing sarcoma (EwS) is an aggressive pediatric cancer of the bone and soft tissues. EwS patients often present with metastatic tumors and have a 5-year survival outcome of 15-30%. Ubiquitin-associated-protein-2-like (UBAP2L) is a well-known stress granule protein and acts as a crucial interactor of Ras-GTPase-Activating Protein SH3-Domain-Binding Protein (G3BP1). Stress granule formation mediated by G3BP1 has previously been linked to sarcoma tumor growth and metastasis. Our research assesses the role of UBAP2L and stress granule formation in metastatic EwS.

Methods: We used the CRISPR-cas9 system to knock out UBAP2L in CHLA10 cells, a metastatic EwS cell line. We have validated UBAP2L protein levels were through Western blotting, and UBAP2L mRNA expression levels were quantified through RT-qPCR. To assess protein stability, we have used cycloheximide chase assay, where we treated cells with 100ug/ml cycloheximide for an indicated period of time. Sodium arsenite was used to induce oxidative stress and stress granule formation. For in vivo studies, we used the murine renal subcapsular EwS model and assessed tumor metastatic potential by observing tumor cell infiltration in the lungs and liver through CD99 immunohistochemistry staining.

Results: UBAP2L is overexpressed in metastatic EwS cell lines, and high mRNA expression of UBAP2L is correlated with poor survival outcomes in Ewing sarcoma patients. CRISPR-mediated knockout of UBAP2L results in inhibition of stress granule formation and sensitizes Ewing sarcoma cells to oxidative stress resulting in increased cell death. Moreover, UBAP2L knock-out cells have significantly reduced stability of G3BP1 protein, both in ambient conditions and under oxidative stress. When CHLA10 cells were treated with MG-132, a proteasome inhibitor, in combination with oxidative stress, G3BP1 protein loss was drastically reduced. Our studies indicate that UBAP2L depletion has no effect on 2D cell culture growth, but drastically reduces cells' ability to form colonies in soft agar. In a renal subcapsular in vivo model, UBAP2L knockout cells formed smaller tumors that were unable to metastasize to the liver and lungs.

Conclusions: Our studies suggest that UBAP2L is highly expressed in metastatic EwS. UBAP2L is necessary for stress granule formation in EwS cells, and knockout of UBAP2L results in cells being unable to survive oxidative stress. We highlight a novel function of UBAP2L as a G3BP1 stabilizer and demonstrate that UBAP2L plays a pro-metastatic role in EwS tumors.

Katlyn Richardson

GRADUATE STUDENT

GRANZYME K: A NOVEL PLAYER IN PSORIASIS DEVELOPMENT

ABSTRACT #2

Background/objectives: Psoriasis is a skin disease that currently affects over one million Canadians. It is characterized by skin inflammation and increased epidermal proliferation forming thick, scaly plaques. Unfortunately, current therapies are not completely effective and often present with side effects. As such, a deeper understanding of the pathological mechanisms of psoriasis is necessary. Granzyme K (GzmK) is a serine protease recently elucidated as a mediator of skin inflammation. Elevated GzmK is observed in human psoriasis skin. However, its role in psoriasis is unknown. We hypothesized that GzmK contributes to the onset and progression of psoriasis through the augmentation of inflammation and/or epidermal proliferation.

Methods: GzmK levels were assessed in skin biopsies from patients with and without psoriasis. The role of GzmK was investigated in an established imiquimod (IMQ)-induced murine model of psoriasis, comparing GzmK knockout (K-KO) to wild-type (WT) mice (n=6 per genotype). IMQ-induced psoriasis severity was assessed for onset and severity of erythema and plaque formation using a modified Psoriasis Area and Severity Index (PASI). IMQ-induced psoriasis tissue extracts were examined histologically and by ELISA for epidermal thickness and relevant pro-inflammatory markers. Cultured macrophages and keratinocytes were treated with GzmK for assessment of pro-inflammatory cytokine expression and epidermal proliferation. To elucidate a mechanism, we are currently defining the GzmK degradome as it pertains to the skin using High-efficiency Undecanal-based N Termini EnRichment (HUNTER), a mass spectrometry-based method for N-termini enrichment from limited samples.

Results: An approximate 40-fold increase (p=0.045) in GzmK was observed in lesional skin from psoriasis patients compared to healthy skin. Mast cells were identified as one of the primary cell sources. IMQ-treated K-KO mice exhibited an average 60% decrease in severity of erythema and plaque formation compared to WT mice (scores of 4.25 vs 7.25 on 8-point modified PASI scale, p≤0.001 at day 7). In histological examination, IMQ-treated K-KO mice had an average 50% decrease (p=0.0053) in inflammatory cell infiltrate compared to WT mice. Pro-inflammatory cytokines, IL-17 and IL-23, commonly elevated in human psoriasis, were also reduced in IMQ-treated K-KO mice. Specifically, IL-17 levels were decreased an average of 46% (2,147 ± 680 vs 4,657 ± 2171 pg IL-17/mg total protein, p=0.044), whereas IL-23 levels were decreased an average of 48% (7,448 ± 1448 vs 15,491 ± 1948 pg IL-23/mg total protein, p<0.001) compared to WT mice, as determined using ELISA. Preliminary in vitro work suggests GzmK-treated macrophages secrete increased levels of IL-23. IMQ-treated K-KO mice also exhibited an average 30% decrease (p=0.014) in epidermal thickness, and 98% decrease (p=0.03) in proliferation markers Ki67 and PCNA, compared to WT mice. In vitro, GzmK induced keratinocyte proliferation as assessed by total cell counts and Ki67 staining (p≤0.002).

Conclusions: GzmK is elevated in human psoriasis tissue and may contribute to psoriasis development. Inhibition of GzmK may represent a novel therapeutic approach for treating psoriasis.

Supervisor: Dr. David Granville

Katlyn Richardson[1,2], Christopher Turner[1,2], Lorenz Nierves[2,3], Rachel Cederberg[2,4], Hongyan Zhao[1,2], Angela Burleigh[2,5], Sho Hiroyasu[1,2], Megan Pawluk[1,2], Layla Nabai[1,2], Kevin Bennewith[2,4,6], Philipp Lange[2,3], Richard Crawford[2,5], David Granville[1,2,7]

Affiliations:
[1]International Collaboration On Repair Discoveries (ICORD), Vancouver Coastal Health Research Institute, University of British Columbia, Vancouver, Canada. [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada. [3]Michael Cuccione Childhood Cancer Research Program, BC Children's Hospital, Vancouver, BC, Canada. [4]Integrative Oncology Department, BC Cancer Research Centre, Vancouver, Canada. [5]Department of Dermatology and Skin Science, University of British Columbia, Vancouver, Canada. [6]Interdisciplinary Oncology Program, University of British Columbia, Vancouver, BC, Canada. [7]British Columbia Professional Firefighters' Burn and Wound Healing Group, Vancouver, Canada.

Emel Islamzada

GRADUATE STUDENT

DEGRADATION OF RED BLOOD CELL DEFORMABILITY DURING STORAGE IN BLOOD BAGS

ABSTRACT #3

Supervisor: Dr. Hongshen Ma

Emel Islamzada[1,2], Kerryn Matthews[2,4], Erik Lamoureux[2,4], Quan Guo[4], Aline T. Santoso[4], Mark D. Scott[1,2,3], and Hongshen Ma[1,2,4,5,6]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC. [2]Centre for Blood Research, UBC. [3]Canadian Blood Services, Ottawa, Canada. [4]Department of Mechanical Engineering, UBC. [5]Department of Urologic Science, UBC. [6]Vancouver Prostate Centre, Vancouver General Hospital

Background/objectives: Red Blood Cell (RBC) transfusions are a life-saving procedure, restoring tissue oxygenation to patients with conditions including hemoglobinopathies and bleeding disorders. However, not all RBC units can deliver the same benefit to the recipients. Donor-to-donor differences in RBC properties lead to differences in RBC storage and affect the post-transfusion circulation time in the recipients. Currently, there is no standardized way to assess the RBC quality directly prior to transfusion. Transfusion of poorly storing units can significantly reduce their clinical benefit, and in some cases cause serious adverse effects.

Our team has developed a unique microfluidic ratchet system which sorts RBCs based on their ability to deform and pass through a series of funnels with opening sizes reflecting the vessel sizes encountered in blood flow. The RBCs are collected in 12 distinct outlets based on deformability, allowing us to obtain a unique deformability signature of each donor sample. We have used this method to show significant differences in RBC deformability profiles between healthy donors in an accelerated blood storage model. We hypothesize that **deformability based sorting of RBCs can be used to measure the quality of donated RBC units**.

Methods: Packed RBCs in standard Fresenius® units collected from healthy donors were obtained from Canadian Blood Services Blood4Research (n=14). The units were stored at approved conditions at 4°C for 8 weeks. RBC deformability and standard hematological parameters (MCV, RDW-CV, MCHC, and MCH) were measured on day 1, week 2, 4, 6, and 8. The deformability of RBC samples is calculated as the cumulative distribution of the RBCs throughout the microfluidic ratchet sorting device, defined as the rigidity score (RS).

Results: RBC units have a mean rigidity score of 3.15±0.37 on the day of collection. Male donors (n=8, 3.00±0.18) show slightly more deformable RBCs on average compared to female donors (n=6, 3.4±0.53) on the first day of storage. Mean RS change throughout the cold storage is 0.30 at week 6 and 0.94 at week 8. While some donors showed a well-conserved deformability profile with modest changes in RS (Donors 2, 3, 4, 6 and 11), other donors showed a marked reduction in deformability (Donors 7, 8 and 10). The hematological parameters stayed mostly within normal range.

Conclusions: Our data demonstrates the microfluidic ratchet device to be a sensitive and robust method of determining individual donor RBC unit deformability signatures. Moreover, our current work on the RBC accelerated storage model suggests the possibility for non-destructive unit quality assessment. The ability to assess the RBC quality directly prior to transfusion has the potential to have a significant impact on transfusion medicine, allowing direct quality control method to eliminate poor storing units. It would additionally allow to match potentially longer storing units with sensitive patients.

Hang Yang

RESIDENT

UHRF1 AS A NOVEL IMMUNOHISTOCHEMICAL MARKER FOR SEPARATING MALIGNANT FROM BENIGN MESOTHELIAL PROLIFERATIONS

ABSTRACT #4

Background/objectives: Differentiating malignant mesotheliomas (MM) from reactive mesothelial proliferations (RMP) on routine histology can be a major diagnostic challenge. UHRF1 is a multidomain protein complex which recruits DNA methyltransferase (DNMT) 1 to newly synthesized DNA, to maintain DNA methylation during mitosis and DNA repair. UHRF1 has been shown to be constitutively overexpressed in numerous malignancies; it contributes to carcinogenesis by methylation and histone modification of tumour suppressor genes, as well as activation of proto-oncogenes and retroviral sequences. UHRF1 expression has been found to be significantly higher in MM relative to normal mesothelial tissue and associated with decreased overall survival in MM. Little is known about expression of UHRF1 in RMP. In this study we asked whether immunohistochemical detection of UHRF1 can be used to separate MM from RMP.

Methods: We optimized UHRF1 anti-human monoclonal antibody for immunohistochemistry, and stained mesothelial microarrays, which contain sarcomatoid/desmoplastic mesotheliomas (SMM), epithelial mesotheliomas (EMM), and reactive mesothelial proliferations with spindled (RMP-S) and epithelial morphologies (RMP-E). We developed a scoring system based on the proportion of cells staining positive, and created a heat map to determine the relationship between UHRF1 positivity and loss of BAP1 or MTAP expression, which are markers of malignant mesotheliomas.

Results: On tissue microarrays, consistent strong uniform nuclear staining of UHRF1 is present in 20/24 SMM, 26/42 EMM, 0/32 RMP-S, and 0/35 RMP-E. The sensitivities for SMM and EMM are 83% and 62%, respectively. The specificities are 100%.

Conclusions: UHRF1 holds great promise as a useful marker to separate malignant mesotheliomas from benign mesothelial proliferations. In the next phase of our study, we will further characterize the staining pattern of UHRF1 by staining whole sections of malignant and benign mesothelial proliferations. We will also examine the relationship between UHRF1 expression and retention or loss of BAP1 and MTAP.

Supervisor: Dr. Andrew Churg

Hang Yang[1], Simon Cheung[2], Andrew Churg[3]

Affiliations:
[1]Department of Pathology, Vancouver General Hospital; Department of Pathology, UBC. [2]Department of Pathology, Vancouver General Hospital. [3]Department of Pathology, Vancouver General Hospital; Department of Pathology, UBC

Julia Naso

RESIDENT

ABSTRACT #5

Supervisor: Drs. Andrew Churg and Stephen Yip

Julia R. Naso[*1], Adrian B Levine[*1], Hossein Farahani[2], Lucian R. Chirieac[3], Sanja Dacic[4], Joanne L. Wright[1,5], Chi Lai[5], Hui-Min Yang[1,6], Steven J.M. Jones[7], Ali Bashashati[1,2], Stephen Yip[**1,6], Andrew Churg[**1,6]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC. [2]School of Biomedical Engineering, UBC Biomedical Research Centre (BRC). [3]Departments of Pathology, Brigham and Women's Hospital and Harvard Medical School, 75 Francis St, Boston, MA, USA. [4]Department of Pathology, University of Pittsburgh, Medical Center PUH C608, 200 Lothrop Street Pittsburgh, PA, USA. [5]Department of Pathology, St Paul's Hospital. [6]Department of Pathology, Vancouver General Hospital. [7]Michael Smith Genome Sciences Centre, BC Cancer Agency.
*These individuals made an equal contribution
** Co-senior authors

DEEP-LEARNING BASED CLASSIFICATION DISTINGUISHES SARCOMATOID MALIGNANT MESOTHELIOMAS FROM BENIGN SPINDLE CELL MESOTHELIAL PROLIFERATIONS

Background/objectives: Sarcomatoid mesothelioma is an aggressive malignancy that can be challenging to distinguish from benign spindle cell mesothelial proliferations based on histology. A deep learning based classifier may be able to aid pathologists in making this critical diagnostic distinction, though no such classifier has previously been developed.

Methods: SpindleMesoNET was trained on unannotated benign spindle cell mesothelial proliferations and malignant sarcomatoid mesothelioma cases with annotated tumor areas. Performance was assessed through cross-validation on the training set, on an independent set of challenging cases referred for expert opinion ('referral' test set), and on an externally-stained test set from outside institutions ('externally-stained' test set).

Results: SpindleMesoNET predicted the benign or malignant status of cases with AUC's of 0.934, 0.925 and 0.989 on the cross-validation, referral and externally-stained test sets, respectively. The accuracy of SpindleMesoNET on the referral set cases (92.5%) was comparable to the average accuracy of three experienced pathologists on the same slide set (91.7%).

Conclusions: We conclude that SpindleMesoNET can accurately distinguish sarcomatoid mesothelioma from benign spindle cell mesothelial proliferations, and holds potential for future use as an ancillary test in diagnostic pathology.

Hezhen Ren

RESIDENT

DIFFERENTIATING MALIGNANT MESOTHELIOMA FROM BENIGN MESOTHELIAL PROLIFERATION USING C-MET IMMUNOHISTOCHEMISTRY

ABSTRACT #6

Background/objectives: The separation of benign from malignant mesothelial proliferations can be challenging for the surgical pathologist. c-MET is a receptor tyrosine kinase that is overexpressed and detectable by immunohistochemistry in many malignancies, including malignant mesothelioma. Whether c-MET is also expressed in benign mesothelial reactions is unclear from the literature. Here, we examined the utility of c-MET immunohistochemistry in differentiating malignant mesothelioma from benign mesothelial proliferations.

Methods: Two tissue microarrays containing 33 reactive epithelioid mesothelial proliferations (E-RMPs), 23 reactive spindle cell mesothelial proliferations, 45 epithelioid malignant mesotheliomas (EMMs), and 26 sarcomatoid/desmoplastic mesotheliomas (SMMs) was stained using c-MET immunohistochemistry. The c-Met result is compared with immunohistochemistry for two established markers, BAP1 and methylthioadenosine phosphorylase (MTAP). Membrane staining for c-MET was evaluated using a 12-point H-score and classified as negative (score = 0), trace (score = 1-3), moderate (score = 4-6), or strong (score = 8-12).

Results: c-MET staining was seen in only 3 of 33 (all trace) E-RMPs compared with 36 of 45 (80%) EMMs (chi-square comparing reactive and malignant = 39.80, p = 1.2 × 10^-8). The H-score was >3 (moderate or strong) in 24 of 45 (53%) EMMs. Addition of BAP1 staining to the c-MET-negative/trace EMM increased sensitivity to 75% (32/42), whereas similar addition of MTAP staining increased sensitivity to 77% (33/43). No benign spindle cell proliferations showed staining compared with 10 of 26 (38%) positive SMMs, but only 4 (15%) SMMs were classified as moderate or strong.

Conclusions: Moderate/strong c-MET staining can be used to support a diagnosis of EMM versus an epithelial reactive proliferation. c-MET is too insensitive to use for detecting SMM

Supervisor: Dr. Andrew Churg

He Zhen Ren[1,2], Simon Cheung[2], Andrew Churg[1,2]

Affiliations:
[1]Department of Pathology, Vancouver General Hospital. [2]Department of Pathology, UBC

Jennifer Ji

GRADUATE STUDENT

ABSTRACT #7

Supervisor: Dr. David Huntsman

Ji JX[1], Cochrane D[2] , Negri GL[3] , Colborne S[3] , Spencer S[3], Farnell D[1], Tessier-Cloutier B[1], Hoang L[1], Mohammad N[1], Huvila J[1], Thompson E[1], Leung S[4] , Chow C[4] , Koebel M[5] , Feil L[6], Anglesio M[6] , Klein Geltink R[1], Goode E[7] , Bolton K[8], Morin G[3], Huntsman DG[1,2,6]

Affiliations:
[1]Department of Pathology and Laboratory medicine, UBC. [2]Department of Molecular Oncology, BC Cancer Agency. [3]Michael Smith Genome Sciences Centre, BC Cancer Agency. [4]Genetic Pathology Evaluation Center. [5]Department of Pathology and Laboratory medicine, University of Calgary. [6]Department of Obstetrics and Gynaecology, UBC. [7]Department of Health Sciences Research, Mayo clinic. 8Department of Medicine, Washington University St. Louis

THE PROTEOMIC AND METABOLOMIC LANDSCAPE OF CLEAR CELL OVARIAN CANCER

Background/objectives: Over 22,440 women were diagnosed with epithelial ovarian carcinoma (EOC) in North America last year. EOC contains subtypes based on histology and prognosis. Each subtype is a distinct disease with unique clinical and molecular features. Despite these differences, the gold-standard treatment for all subtypes remains platinum-based chemotherapy. Late stage clear cell ovarian cancer (CCOC) is not responsive to standard chemotherapy which results in suboptimal outcome for patients. In-depth molecular insight beyond genomic alterations is urgently needed to stratify the disease and drive therapeutic development. In this study, we conducted global proteomics in 192 cases of CCOC comparing to other epithelial ovarian cancer subtypes including high grade serous (HGSC) and endometrioid ovarian cancers (ENOC). In addition, metabolomic study was completed on a smaller cohort to identify metabolic alterations and facilitate integration with proteomic data.

Methods: We collected a large cohort of archival ovarian cancer tumours through local and Canada-wide tumour banks. CCOC cases were reviewed by trained pathologists. The cases with highest quality tumours were chosen for proteomic analysis. Using SP3-CPT, a mass spectrometry-based technique, we characterized the global proteome of CCOC (n = 192), ENOC (n = 35), and HGSC (n=34). We identified differentially expressed proteins between ovarian cancer subtypes Protein Expression Control Analysis R package. Targets were validated using immunohistochemistry on clinical samples using local tissue microarrays. In order to identify CCOC subtypes within the tumor samples, we applied Consensus Clustering with K-means algorithm, using the top 50% most variably expressed proteins. Metabolomics screening was completed on a subset of the proteomic cohort using frozen tissue.

Results: CCOC had a distinct proteome compared to other EOC subtypes based on principal component analysis and hierarchical clustering. Proteomic analysis identified previous CCOC specific clinical and biological markers. Our cohort of CCOC was split into 4 subgroups based on their proteomic signatures, including the cellularity based fibrotic and necrotic subgroups, and the biology-based coagulation and metabolic subgroups. In a survival analysis, the metabolic subgroup showed better progression-free and overall survival compared to the rest of the groups, whereas coagulative subgroup had the worst progression-free and overall survival. Several metabolic pathways are highlighted by proteomics and metabolomics analysis, including the purine metabolism pathway. Immunohistochemistry validation confirms the unique expression of guanine deaminase in CCOCs, and further in vitro experiments are on-going.

Conclusions: In this study, we profiled the global proteomic and metabolomic landscape of CCOC, compared to other EOC subtypes ENOC and HGSC. Our study provided new insights into CCOC biology. Furthermore, we show that CCOC is a heterogenous disease with distinct metabolic enzyme alterations. The results of this study provide targetable pathway candidates for further therapeutic development.

Elyn Rowe

GRADUATE STUDENT

PERIPHERAL APOLIPOPROTEIN E: OVERLOOKED IN THE CONTEXT OF ALZHEIMER DISEASE?

ABSTRACT #8

Supervisor: Dr. Cheryl Wellington

Elyn M. Rowe[1], Yoshihiro Kaneoya[2] , Ging-Yuek Robin Hsiung[3] , Cheryl L. Wellington[1]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC. [2]Division of Research and Development, Denka Co. Ltd, Gosen-City, Niigata, Japan. [2]Division of Neurology, UBC

Background/objectives: Late-onset Alzheimer disease (LOAD) is the most common cause of dementia. The cerebrovasculature plays a central role in maintaining brain health and considerable evidence suggests that cerebrovascular dysfunction is an initiating event in the LOAD pathology. High-density lipoproteins (HDL) are circulating particles known to protect and reinforce the vasculature, making them of interest in this context. The standard clinical measure of HDL's cholesterol content (HDL-C) has been assessed as a LOAD biomarker with inconsistent results. However, HDL-C is a crude measure of complex and heterogeneous HDL particles. HDL containing apolipoprotein E (ApoE-HDL) is especially cardioprotective and measures of ApoE-HDL outperform HDL-C as a heart disease biomarker. As the APOE4 variant is the main genetic risk factor for LOAD, ApoE-HDL may also be a useful biomarker for LOAD. This pilot study aimed to: 1) Determine whether ApoE-HDL levels associate with APOE genotype, and 2) Determine whether ApoE-HDL is a better predictor than standard lipid measures as a diagnostic LOAD biomarker.

Methods: A clinic-based pilot study was performed with 175 cases of clinically-diagnosed LOAD and 30 cognitively normal controls. Participants were not age- or sex-matched due to limited sample availability. A novel assay that selectively measures cholesterol from HDL containing ApoE (ApoE-HDL-C) was used to quantify ApoE-HDL in banked serum samples, and a standard lipid panel (HDL-C, total cholesterol, LDL-C) was also measured. Univariable logistic regression was used to measure the discriminative capacity of ApoE-HDL-C and standard lipid measures for clinical LOAD diagnosis.

Results: ApoE-HDL-C levels are significantly higher in females ($p<0.0001$), are not associated with age (rho = -0.043, p = 0.397), and are not associated with APOE genotype (p =0.608). Due to a significant bias toward females in the control group (83.3% vs 58.9% in the case group) and the strong association of ApoE-HDL levels with sex, preliminary univariate analyses to investigate ApoE-HDL-C as a diagnostic biomarker was only done in females. Discriminative capacity of ApoE-HDL for clinical LOAD diagnosis was weak (AUC = 0.631) but outperformed all other lipid measures. The unit odds ratio for ApoE-HDL as a predictor of LOAD diagnosis in females was 0.778 (p = 0.018) for ApoE-HDL, indicating that lower levels are associated with increased odds of a clinical LOAD diagnosis.

Conclusions: APOE genotype does not mediate ApoE-HDL levels as measured by cholesterol content. However, APOE4-carriers may have more cholesterol-rich ApoE-HDL than non-carriers, which would mask a genotype effect when measured with this assay. Given the weak discriminative capacity of ApoE-HDL-C for disease diagnosis in females, these results do not support its use as a diagnostic biomarker. Multivariable analysis is planned to further explore the relationship of ApoE-HDL-C with clinical LOAD diagnosis in the context of relevant co-variates. Future directions include evaluating ApoE-HDL-C as a prognostic biomarker for LOAD in a longitudinal cohort, and exploring other methods to quantify ApoE-HDL particles.

Kevin Kuchinski

GRADUATE STUDENT

RECOVERING INFLUENZA GENOMES FROM WILD BIRD HABITATS FOR OUTBREAK PREVENTION AND PANDEMIC PREPAREDNESS

ABSTRACT #9

Supervisor: Dr. Natalie Prystajecky

Kevin Kuchinski[1], Jun Duan[1], Michelle Coombe[2,3], Chelsea Himsworth[2,4,5], William Hsiao[1,6], Natalie Prystajecky[1,7]

Affiliations:
[1]Department of Pathology and Laboratory Medicine; UBC. [2]School of Population and Public Health; UBC. [3]Regulatory Unit; British Columbia Ministry of Agriculture, Food, and Fisheries; Abbotsford. [4]Animal Health Centre; British Columbia Ministry of Agriculture, Food, and Fisheries; Abbotsford. [5]Canadian Wildlife Health Cooperative; Abbotsford. [6]Faculty of Health Sciences; SFU; Burnaby. [7]Public Health Laboratory; BCCDC

Background/objectives: Avian influenza viruses (AIVs) cause severe disease in poultry and dangerous zoonotic infections in humans. Poultry outbreaks are costly, and they allow novel flu viruses to spill-over into human hosts, threatening future pandemics and new types of seasonal flu. A cornerstone of outbreak prevention and pandemic preparedness is AIV surveillance in wild waterfowl, the virus' natural animal reservoir. Traditional surveillance involves swabbing birds and culturing viruses to identify strains from viral subtypes associated with high pathogenicity. This process is time-consuming, and it suffers from limited and biased sampling of the local waterfowl community. It rarely provides advance warning of dangerous strains before they cause outbreaks. We have developed an alternative, culture-independent method for detecting and subtyping AIVs using targeted genomic sequencing. In addition to traditional swab specimens, our approach can be applied to environmental specimens from wild bird habitats, specifically wetlands sediment where virus-laden feces from numerous bird species accumulates. We investigated whether these specimens allow extensive identification of locally circulating AIV strains.

Methods: Sediment specimens and bird swabs were collected from wetlands around the Lower Mainland and Fraser Valley of British Columbia between September 2016 and April 2018. Total RNA was extracted from each sample and screened for AIV genomic material by qPCR. 240 qPCR-positive samples (184 sediments and 46 swabs) were prepared into metagenomic sequencing libraries using a custom protocol. Hemagglutinin (HA), neuraminidase (NA), and matrix (M) segments of the influenza A virus genome were captured in these libraries using 3,600 custom hybridization probes then sequenced on an Illumina HiSeq. Genomic sequencing results were analyzed with custom bioinformatic pipelines to identify AIV sequencing reads, determine their HA and NA subtypes, and assess sequences from H5 and H7 subtypes for the presence of genetic markers associated with high pathogenicity.

Results: AIV genomic material was weakly detected by qPCR in these sediment and swab specimens (Ct value median: 33.5, IQR: 31.0 - 36.2). Using our custom targeted genomic sequencing method, AIV genomic sequences were recovered from 107 sediment specimens (58%) and 25 swab specimens (54%). 13 of 16 avian HA subtypes were detected in sediment while 7 were detected in swabs. All 9 avian NA subtypes were detected in sediment while 6 were detected in swabs. Subtypes H5 and H7, which are associated with highly pathogenic outbreaks, were detected in 9 specimens. Sequencing reads spanning the virulence-determining HA cleavage site were found in one of these specimens. All reads described canonical low-pathogenicity cleavage motifs.

Conclusions: Targeted genomic sequencing allows for culture-independent AIV detection and subtyping in both swab and complex environmental specimens with a sensitivity comparable to traditional culture-based methods. A broad diversity of HA and NA subtypes were recovered in sediment specimens, demonstrating the utility of environmental sampling for AIV surveillance.

Jonathan Lee

RESIDENT

CD34 STAINING AS A USEFUL TOOL IN DISORDERS OF COLLAGEN DEGENERATION

ABSTRACT #10

Background/objectives: The human progenitor-cell antigen CD34 is expressed in dermal dendritic cells and is lost in several disorders affecting dermal collagen. The loss of CD34 immunohistochemical staining has been demonstrated to be helpful in the histologic diagnosis of morphea, lichen sclerosus and the classic pattern of granuloma annulare. The present study characterized CD34 expression in two sclerosing disorders affecting the subcutis: lipodermatosclerosis (LDS) and the sclerodermoid form of chronic graft-versus-host disease (ScGVHD). Additionally, we applied CD34 staining to the interstitial pattern of granuloma annulare (IGA), which is a diagnostically challenging entity with subtle amounts of dermal collagen degeneration.

Methods: Skin biopsies from cases of LDS, ScGVHD, and IGA were collected and reviewed for diagnostic accuracy. The cases were stained with CD34, and staining intensity was graded as either normal, diminished, or absent. The extent and pattern of altered staining was noted for each case, with unaffected areas of dermis serving as an internal positive control. Since LDS and ScGVHD prominently affect the subcutis, CD34 staining was performed on a skin biopsy with normal subcutaneous tissue as a normal control.

Results: 15 cases of LDS, 6 cases of ScGVHD and 4 cases of IGA were identified and stained with CD34. All cases of LDS showed loss of CD34 within subcutaneous septa, and 9 cases (60%) also exhibited full-thickness dermal loss of interstitial staining. All 6 cases of ScGVHD showed varying degrees of CD34 loss within the dermis and/or subcutaneous septa. The normal subcutis showed diffuse septal staining with CD34, with a density equal to that seen in the dermis. CD34 staining was lost in areas of dermal inflammation in half of the IGA cases.

Conclusions: We conclude that CD34 staining is a useful ancillary test in disease processes affecting the subcutaneous collagen such as LDS and ScGVD. Its utility also extends to diagnostically challenging disorders of dermal collagen degeneration such as IGA.

Supervisor: Dr. Richard Crawford

Jonathan M.C. Lee[1,2], Lisa J. Borretta[1,2], Richard I. Crawford[1,2]

Affiliations:
[1]Department of Pathology & Laboratory Medicine, UBC.
[2]Department of Pathology & Laboratory Medicine, Vancouver General Hospital

Yi Ariel Liu

RESIDENT

ABSTRACT #11

Supervisor: Dr. Richard I. Crawford

Deidre Ongaro[1,2], Yi Ariel Liu[1,2], Richard I. Crawford[1,2]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Pathology and Laboratory Medicine, Vancouver General Hospital

BASOPHILIC GEL-LIKE ARTIFACT ORIGINATING FROM FORMALIN-ABSORBENT PADS AND ITS MIMICRY OF FOREIGN MATERIALS IN PATIENT SPECIMENS

Background/objectives: Formalin-absorbent pads reduce exposure to formalin fumes and are widely used in surgical pathology laboratories. We report a basophilic gel-like artifact originating from formalin-absorbent pads which mimics the morphology of various substances found in patient specimens.

Methods: Basophilic gel artifact was identified in 54 hematoxylin and eosin (H&E) stained sections from 15 dermatopathology cases collected prospectively during an eight week period. To further characterize the artifact and its potential mimicry, a panel of histochemical stains was performed on the absorbent pad material, hyaluronic acid soft tissue filler (Juvéderm®), sodium polystyrene sulphate (kayexelate) and patient specimens containing myxoid and chondroid matrix, calcium and mucin.

Results: Affected cases were mostly resection specimens (13/15 cases and 2/15 biopsies). The artifact was located at peripheral surfaces of tissue sections, although in 6 of 54 blocks (11%) there was significant tissue overlap ranging from 1.0 to 5.5 mm from tissue edges. The artifact was present in contiguous levels in 2 of 54 blocks, confirming that the substance was embedded in some of the paraffin blocks. None of the affected cases were accompanied by a history of soft tissue fillers.

Morphologic appearances were indistinguishable from hyaluronic-acid soft tissue fillers and displayed features overlapping with gastrointestinal medication resins, myxoid matrix, calcium and mucin. The artifact stained bright blue with Alcian blue 2.5 (AB) and was non-digestible with hyaluronidase, did not stain with Periodic acid-Schiff (PAS) and was pale magenta with mucicarmine. Hyaluronic-acid soft tissue filler was also bright blue with AB but digestible with hyaluronidase, deep violet with PAS and bright magenta with mucicarmine.

Kayexalate was morphologically distinguishable as small angulated fragments with a compact regular scaled pattern that are pale with AB, green-to-black with Ziehl-Neelsen (formalin pad: deep blue) and magenta with PAS. By contrast, the embedded formalin pad artifact generally has larger fragments with a globular morphology and a loose folded pattern of irregular ‘scales’.

Conclusions: Pathologists should be aware of this artifact, particularly when reviewing small biopsies, and should consider the contextual, morphologic and histochemical staining properties that can assist in distinguishing this basophilic artifact from materials found in patient specimens. Techniques to avoid introducing artifactual material, such as use of a barrier, are recommended when prosecting specimens where there is a higher risk of contamination and misinterpretation.

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Bradley Chaharyn

RESIDENT

SMALL VESSEL VASCULITIS IN BIOPSIES OF ANTI-MYELIN OLIGODENDROCYTE GLYCOPROTEIN ENCEPHALITIS

ABSTRACT #12

Background/objectives: We report the neuropathology of two pediatric brain biopsy cases associated with serum anti-myelin oligodendrocyte glycoprotein (MOG) positivity. Descriptions of anti-MOG associated neuropathology are limited, with initial reports describing various patterns of inflammatory demyelination. Our first patient presented with confusion, speech abnormalities and personality changes following a treated streptococcus throat infection. Our second patient had a past medical history of neurofibromatosis type 1 (NF1) and presented with hypersomnolence and focal neurological deficits. MRI abnormalities included diffuse scattered T2 FLAIR hyperintensities +/- enhancement. CSF was positive for anti-MOG antibodies in both cases, while one case exhibited additional anti-NMDA-R antibodies. Brain biopsies revealed vasocentric mononuclear inflammation featuring a predominance of lymphocytes that included intramural forms, as well as diffuse microglial activation, but no evidence of microglial nodules or microorganisms. One case demonstrated mild perivascular demyelination. The prevailing pattern in both cases was suggestive of “small vessel childhood primary angiitis of the central nervous system” (SVcPACNS). Our results parallel recent reports of anti-MOG neuropathology describing small vessel vasculitis, contrary to initial and subsequent reports that describe “encephalitis”. The foregoing suggests that the neuropathology associated with serum anti-MOG positivity may be broader than first appreciated. Moreover, this pattern of vasculitis might have implications for the natural history of this nascent disorder.

Supervisor: Dr. Chris Dunham

Bradley M. Chaharyn[1] and Christopher P. Dunham[1]

Affiliations:
[1]Division of Neuropathology, University of British Columbia, Vancouver, BC

Sam Chorlton

RESIDENT

PREDICTION OF CYTOMEGALOVIRUS ANTIVIRAL RESISTANCE USING NEXT-GENERATION SEQUENCING IN A CLINICAL VIROLOGY LABORATORY

ABSTRACT #13

Supervisor: Dr. Chris Lowe

Samuel D. Chorlton[1], Gordon Ritchie[1,2], Tanya Lawson[1,2], Elizabeth McLachlan[3], Marc G. Romney[1,2], Nancy Matic[1,2], Christopher F. Lowe[1,2]

Affiliations:
[1]University of British Columbia. [2]St. Paul's Hospital. [3]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada

Background/objectives: CMV infection causes a large burden of disease in immunocompromised patients, and antiviral drug resistance (AVDR) testing is critical to guide management in those patients with antiviral treatment failure. We developed a next-generation sequencing (NGS) assay for CMV AVDR genotyping, enabling faster turnaround time.

Methods: We performed a retrospective review of CMV AVDR testing requests at our laboratory between 2017 and 2019. For the NGS approach, amplicons were generated of UL97 and UL54, sequenced on a MinION sequencer, and analysed with a novel cloud bioinformatics pipeline (BugSeq). Results were compared to traditional Sanger sequencing at the national reference laboratory.

Results: Twenty patient samples were included in analysis and sequenced on a MinION. NGS had 100% sensitivity for AVDR variants detected with Sanger sequencing. Six samples had no AVDR mutations detected using either NGS or Sanger. In the remaining 14 samples, NGS identified additional mutations conferring AVDR in UL97 and/or UL54 in 8 (57%) samples as compared to Sanger sequencing. These mutations were minority variants present in 10-30% of NGS reads.

Conclusions: Amplicon NGS using a MinION enables accurate AVDR prediction for CMV resistance. Pairing this technology with a novel cloud platform can potentially enable NGS in clinical laboratories for rapid AVDR genotyping.

Michael Multan

RESIDENT

AUTOPSY EDUCATION IN CANADIAN PATHOLOGY PROGRAMS: A SURVEY OF CANADIAN TRAINEE (Recently published: The American Journal of Forensic Medicine and Pathology: January 21, 2021 - Volume Publish Ahead of Print - Issue - doi:10.1097/PAF.0000000000000665)

Background/objectives: This survey of Canadian pathology residents was designed to quantify the number of autopsies Canadian residents aim to complete during residency training, to better understand the perception of residents about access and quality of autopsy skills education. In addition, the interest of current pathology residents in autopsy and forensic pathology as a future career was also assessed.

Methods: A web-based survey was sent to all Canadian pathology residents. This survey consisted of 19 questions on institution, level of training, intention to complete the American Board of Pathology examination, number of autopsies completed, perception of quality/access to autopsy skills education, interest, and factors contributing to autopsy and forensic pathology.

Results: Eighty two (26%) of a possible 310 residents (12/47 general pathology, 70/263 anatomical pathology) across all Canadian institutions offering anatomical or general pathology programs (16/16 institutions) participated in the survey. Eighty-three percent of the respondents rated autopsy education as either very important or important. Fifty-five percent of the respondents intended to either challenge the American Board of Pathology examination or wanted the option to do so in the future, whereas only 47% of the participants agreed that all residents who wish to challenge the examination will easily be able to complete 50 autopsies during residency. Only 18% of the respondents were interested in performing autopsies as a major part of their career, and a combined 52% were only interested in performing autopsies to secure a desired position or felt that having to do autopsies would be a job deterrent. The quality of autopsy teaching received and the number of autopsies performed was identified as the most significant factors affecting interest in performing autopsies as part of a future career. A combined 68% of the respondents felt that the job market in forensic pathology in Canada was either good (better than most subspecialties) or very good (more jobs than graduating fellows). Seventy-one percent (12/17) of postgraduate year 5 respondents reported having completed 50 or more autopsies at the time of survey completion. Eleven percent of the respondents did not agree that all residents who document having completed an autopsy at their institution will have participated in all 8 essential autopsy tasks. Twenty four (29%) of the 82 respondents provided detailed narrative comments.

Conclusions: Most Canadian pathology residents believe that autopsy education is an important component of residency training. Limited access to quality autopsy teaching seems to be an important factor in resident interest in forensic pathology as a future career, despite a perceived good job market in comparison with most other subspecialties.

ABSTRACT #14

Supervisor: Dr. Steven White

Michael Multan[1], Pauline Alakija[2], Matthew Orde[1], Steven White[1]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Laboratory Medicine & Pathology, University of Alberta, Edmonton, Alberta

Ann Tran

RESIDENT

ABSTRACT #15

Supervisor: Drs. Vilte Barakauskas and Kate Chipperfield

Ann Tran[1], Salina Kung[1], Yasmin Derayat[1], Alastair Williams[1], Veena Lin[1], Vivienne Beard[1], Charles Cochrane[1], Hannah Foggin1, Horacio Osiovich[1,2], Robert Everett[1,3], Leanne Dahlgren[1,4], Wee-Shian Chan[1,5], Nick Au[1,6], Karla Muyzer[7], Catherine Leung[7], Michelle Dittrick[6], Ashton Ellis[6], Kate Chipperfield[1,6], Vilte Barakauskas[1,6]

Affiliations:
[1]Faculty of Medicine, UBC. [2]Department of Pediatrics, BC Children's Hospital. [3]Department of Neonatology, BC Children's Hospital. [4]Department of Obstetrics, BC Women's Hospital. [5]Department of Medicine, BC Women's Hospital. [6]Department of Pathology and Laboratory Medicine, BC Children's and Women's Hospital. [7]Patient Co-Lead, BC, Canada

PLANNING AND PRELIMINARY RESULTS FOR P2RISM: PEDIATRIC AND PREGNANCY REFERENCE INTERVALS FOR SAFE MEDICINE

Background/objectives: Reference intervals (RIs) accompany lab results to aid clinicians in result interpretation and decision-making. RIs represent the central 95% of normal values derived from testing a healthy population and should be established by individual labs. This undertaking is not routinely carried out due to its resource intensiveness. Accurate RIs applicable to Canadian pregnant women and neonates are particularly lacking as recruiting healthy people to donate blood samples, especially children, is challenging. We aimed to recruit healthy post-partum women and neonates for a priori establishment of RIs for common lab tests and to gain insights into participant experiences via surveys.

Methods: We worked with multiple stakeholders (patient leads, nurses, laboratory assistants) to establish patient-centered methods for recruiting and collecting blood from healthy post-partum women and neonates in order to establish RIs for biochemical, hematologic, and coagulation tests specific to these populations. We also collected participant feedback at the time of consent and end of participation to guide future RI studies at other gestational and pediatric time points.

Results: After 12 total months of enrolment, 38% (91/235) of women who engaged with the study have consented, with helping healthcare, science, and future patients being predominant reasons for participating. Women who choose not to donate blood can anonymously provide feedback about reasons for declining. Of the 39 decline surveys submitted, women mostly stated they were too tired, busy, or overwhelmed to participate.

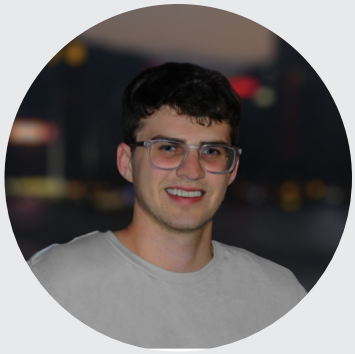
Approximately one-third of moms (30%, 24/79) were deemed ineligible through review of their health questionnaire and/or medical chart and an additional 25% (20/79) could not have blood drawn due to logistical factors. Neonates had similar ineligibility rates with a larger proportion of uncollected neonatal samples due to logistical or technical factors (48%, 31/64). While nearly all samples collected from moms were of acceptable quality, approximately 50% of neonatal hematologic and coagulation samples and 70% of chemistry samples met acceptability criteria.

At the time of analysis, 48 participants completed the follow-up surveys and nearly all (94%, 45/48) stated they would recommend study participation to a friend. The main factors women felt would help future families participate in similar studies included having no extra hospital trips, having online surveys, and ensuring families were well prepared for the blood collection.

Conclusions: The preliminary P[2]RISM results suggest mothers who participate in research are motivated by altruistic reasons. Factors that may help future participation in blood collection studies in healthy pediatric populations reflect convenience and education. The feasibility of successful RI studies involving pregnant women and neonates requires large recruitment numbers, as a predominant proportion of consented participants may be excluded from RI calculation due to ineligibility, logistical factors, or sample quality concerns.

Brett Collinge

GRADUATE STUDENT



THE MUTATIONAL LANDSCAPE OF DOUBLE/TRIPLE-HIT HIGH-GRADE B-CELL LYMPHOMA WITH BCL2 REARRANGEMENT (DH/TH-BCL2)

ABSTRACT #16

Background/objectives: The poor outcome of high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangement (DH/TH) highlights the need for identifying specific targetable biology. Although a gene expression signature (DHITsig) shared by DH/TH-BCL2 tumours has confirmed a unified biology, the genetic features of these tumours beyond MYC/BCL2 rearrangement are not well established. Here, we explore the mutational landscape of DH/TH-BCL2 to identify key molecular features that may inform on targetable tumour cell vulnerabilities.

Methods: Diagnostic biopsies of 102 DH/TH-BCL2 tumours (morphology: 60 diffuse large B-cell lymphoma (DLBCL); 42 high-grade – intermediate or blastoid) were identified with break-apart fluorescent in situ hybridization (FISH). Tumours with DLBCL morphology had no history of indolent lymphoma and were sourced from BC Cancer where unbiased FISH testing was applied. Among tumours of high-grade morphology, 29% had a history of indolent lymphoma. High confidence somatic mutations (SNVs/Indels) were identified on whole genome or exome sequencing using an ensemble of variant callers (Strelka2, LoFreq, Mutect2, SAGE). Mutation frequencies among recurrently mutated genes were compared to Burkitt lymphoma (BL; Grande et al. Blood 2019) and DHITsig-neg DLBCL (Schmitz et al. NEJM 2018) to identify genes with significant differences in the recurrence of non-silent mutations (FDR < 0.05). Genetic subtypes were assigned using the LymphGen algorithm.

Results: The most frequently mutated genes in DH/TH-BCL2 were in chromatin modifiers KMT2D, CREBBP, and EZH2, along with BCL2 and TNFRSF14 – genes commonly mutated in follicular lymphoma (FL) and DLBCL with the EZB genetic subtype. Consistently, 92% of tumours were classified into the EZB genetic subtype by LymphGen. Comparisons of DH/TH-BCL2 to DHITsig-neg DLBCL revealed less frequent alterations of genes involved in NF-κB signaling and immune surveillance. Mutations in germinal centre dark zone (DZ) regulatory genes FOXO1 and CCND3 were more frequent in DH/TH-BCL2 – genes that are also recurrently mutated in BL. While mutations in NF-κB signaling pathways and immune surveillance were also uncommon in BL, these tumours were readily distinguishable from DH/TH-BCL2 by the absence of “FL-like” mutations and more frequent alterations to ID3, DDX3X, FBXO11, ARID1A, and SMARCA4.

Conclusions: The similarity to FL is suggestive of an evolutionary trajectory through an FL/FL-like common progenitor, where DH/TH-BCL2 diverges from this trajectory by adopting a DZ-like identity. This is supported by near universal DHITsig positivity of both DH/TH-BCL2 and BL, suggesting this signature more accurately identifies a DZ biology that may be at least partly promoted by MYC rearrangement and mutations in FOXO1 and CCND3. Infrequent mutations promoting immune escape in DH/TH-BCL2 and BL is consistent with a decreased selective pressure due to the “immune cold” nature of the DZ. This unique biology identified among DH/TH-BCL2 tumors provides a framework for future biomarker development as well as rationale for considering potential targeted therapeutic candidates, such as EZH2 inhibitors.

Supervisor: Dr. David Scott

B.J. Collinge[1], L.K. Hilton[1], J. Wong[1], S. Ben-Neriah[1], W. Alduaij[1], C.K. Rushton[2], G. W. Slack[1], P. Farinha[1], T. Miyata-Takata[3], J. R. Cook[4], G. Ott[5], A. Rosenwald[6], E. Campo[7], C. Amador[8], T.C. Greiner[8], P.W. Raess[9], J.Y. Song[10], G. Inghirami[11], E.S. Jaffe[12], D.D. Weisenburger[10], W.C. Chan[10], H. Holte[13], K. Beiske[14], K. Fu[15], J. Delabie[16], S. Pittaluga[12], A.L. Feldman[17], L.H. Sehn[1], K.J. Savage[1], A. J. Mungall[18], L.M. Staudt[19], C. Steidl[1], L.M. Rimsza[20], R.D. Morin[2], D.W. Scott[1]

Affiliations:
[1]BC Cancer, Centre for Lymphoid Cancer. [2]SFU, Molecular Biology and Biochemistry, Burnaby, Canada. [3]Niigata University Graduate School of Medical and Dental Sciences, Div. of Molecular and Cellular Pathology, Niigata, Japan. [4]Cleveland Clinic, Dept. of Molecular Pathology and Laboratory Medicine, Cleveland, Ohio, USA. [5]Robert-Bosch-Krankenhaus and Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Dept. of Clinical Pathology, Stuttgart, Germany. [6]University of Wuerzburg, Institute of Pathology, Wuerzburg, Germany. [7]Hospital Clinic of the University of Barcelona, Dept. of Pathology, Barcelona, Spain. [8]University of Nebraska Medical Center, Dept. of Pathology and Microbiology, Omaha, Nebraska, USA. [9]Oregon Health & Science University, Dept. of Pathology, Portland, Oregon, USA. [10]City of Hope National Medical Center, Dept. of Pathology, Duarte, California, USA. [11]Weill Cornell Medicine, Pathology and Laboratory Medicine, New York, New York, USA. [12]National Cancer Institute, Laboratory of Pathology, Bethesda, Maryland, USA. [13]Oslo University Hospital, Dept. of Oncology, Oslo, Norway. [14]Oslo University Hospital, Dept. of Pathology, Oslo, Norway. [15]Roswell Park Cancer Institute, Dept. of Pathology & Laboratory Medicine, Buffalo, New York, USA. [16]University Health Network and University of Toronto, Dept. of Laboratory Medicine and Pathobiology, Toronto, Canada. [17]Mayo Clinic College of Medicine, Laboratory Medicine and Pathology, Rochester, Minnesota, USA. [18]BC Cancer, Canada’s Michael Smith Genome Sciences Centre, Vancouver, Canada. [19]National Cancer Institute, Center for Cancer Research, Bethesda, Maryland, USA. [20]Mayo Clinic, Dept. of Laboratory Medicine and Pathology, Scottsdale, Arizona, USA

Han Qi Zhao

GRADUATE STUDENT

ABSTRACT #17

METABOLOMIC ANALYSIS OF COLD-STORED PLATELETS

Supervisor: Dr. Dana Devine

Hanqi (Wayne) Zhao[1,2], Katherine Serrano[1,2,3], Angelo D'Alessandro[4] and Dana V. Devine[1,2,3]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC. [2]Centre for Blood Research, UBC. [3]Centre for Innovation, Canadian Blood Services. [4]Department of Biochemistry and Molecular Genetics, University of Colorado Denver – Anschutz Medical Campus, Aurora, Colorado, USA

Background/objectives: Platelet concentrates are currently stored at room temperature under constant agitation for up to 5-7 days depending on national regulations. However, platelet quality deteriorates during storage and room temperature storage also increases the risk of bacterial growth. Cold-stored platelets have the potential to extend storage by reducing bacterial growth and preserve platelet quality. It has been shown that storage temperature has a dramatic effect on platelet metabolites which are the intermediates of platelet functions. While previous research has extensively studied the in vitro storage characteristics of platelets, few have studied the metabolic changes of platelets stored at different temperature. In this study, we explore the metabolomic changes of platelets stored at different temperatures. We hypothesize that CPs will have a distinct metabolomic profile compared to RPs.

Methods: Platelet concentrates were produced from whole blood using the buffy coat method. Per biological replicate, three platelet units were pooled together and split into three identical storage bags. Units were either placed at 22°C with constant agitation (RPs), or 4°C with agitation (CPAs) or 4°C without agitation (CPs). Platelets were storage for 14 days. Platelets were sampled on day 1,2,7 and 14 and metabolites were analyzed by liquid chromatography and tandem mass spectroscopy. A total of 8 biological replicates were performed. One-way ANOVA was used to compare the difference between the metabolite mass spectrometry peak intensities of platelets stored in the three conditions. Data were shown as the mean±1 standard deviation. Multiple comparisons were corrected by the Bonferroni test.

Results: Storage temperature had significant impact on the metabolite level of stored platelets. Cold storage significantly preserved the physiological energy of platelets. For example, there was a significant decrease of glucose level in RPs ($3.65\times10^{7}\pm2.18\times10^{6}$) compared to CPAs ($5.84\times10^{7}\pm2.99\times10^{6}$) and CPs ($5.90\times10^{7}\pm2.35\times10^{6}$) after 14 days of storage suggesting higher consumption of biological energy in RPs($P<0.0001$). Furthermore, cold storage also maintained a consistent level of antioxidants to combat oxidative stress compared to room-temperature storage. Glutathione, which is a known antioxidant was significantly lower ($P<0.0001$) in RPs ($5.76\times10^{4}\pm3.73\times10^{4}$) compared to CPAs ($2.85\times10^{5}\pm5.82\times10^{4}$) and CPs ($3.5\times10^{5}\pm6.18\times10^{4}$) at the end of storage period.

Conclusions: Agitated and non-agitated cold-stored platelets have more energy reserve and less oxidative stress compared to the standard room-temperature stored platelets. These findings suggest that cold storage significantly preserves the metabolic potential of platelets. Together with the added benefit of reduced bacterial growth, cold-stored platelets are a promising transfusion product for patients.

Rachel Cederberg

GRADUATE STUDENT

EOSINOPHILS KILL TUMOR CELLS VIA DEGRANULATION AND DECREASE PULMONARY COLONIZATION BY METASTATIC MAMMARY

ABSTRACT #18

Background/objectives: The immune system is an integral component of both the primary tumor and distal, pre-metastatic sites, where host immune cells can contribute to both tumor progression and anti-tumor immunity. The lungs, which are one of the most common sites of breast cancer metastasis, are host to a variety of immune cell subsets, including eosinophils (Eo). Eo are innate immune cells that target pathogens via the secretion of cytotoxic granule proteins (degranulation), such as eosinophil peroxidase. Similarly to other innate immune cells, Eo have been shown to be anti-tumorigenic when exposed to certain signals from their local microenvironment. For example, metastatic melanoma patients with elevated circulating Eo respond better to immunotherapy, and Eo have been shown to directly kill tumor cells. We have found that transgenic mice that over-express the cytokine IL-5 (IL5Tg mice), a critical protein for Eo development and survival, have a 100-fold expansion of Eo in the lungs and are protected from breast cancer pulmonary colonization. We hypothesize that Eo impair pulmonary metastasis.

Methods: We used IL5Tg (exhibit eosinophilia) and dblGATA (Eo-deficient) transgenic mice to study pulmonary metastasis. EO771 breast cancer cells were injected intravenously (i.v.) to seed the lungs. Eo were also co-cultured ex vivo with tumor cells and Eo-mediated tumor cell death was quantified by flow cytometry. Eo degranulation was measured with a colorimetric eosinophil peroxidase activity assay.

Results: We confirmed that IL5Tg mice have a systemic expansion of Eo and significantly lower EO771 mammary carcinoma pulmonary tumor burden compared to wild-type (WT) mice. We found that Eo-deficient dblGATA mice exhibited accelerated metastatic progression compared to both WT and IL5Tg mice injected i.v. with EO771 cells. We also found that IL5Tg mice had a decreased frequency and total number of EO771 tumor cells compared to WT and dblGATA mice 5 days post-i.v. injection, indicating that Eo may play a role in both initial tumor cell colonization and subsequent metastatic nodule progression. Importantly, we found that Eo isolated from naïve IL5Tg mice were able to directly kill both EO771 and Lewis lung carcinoma (LLC) tumor cells ex vivo. Additionally, Eo co-cultured with tumor cells released eosinophil peroxidase, a cytotoxic granule protein, indicating that Eo were actively degranulating. These data identify degranulation as a potential mechanism by which Eo can kill tumor cells and inhibit pulmonary metastatic colonization of EO771 mammary carcinoma cells.

Conclusions: Once breast cancer metastasizes, it becomes incredibly challenging to treat, highlighting the need for a better understanding of host factors that prevent metastasis, as well as improved therapeutics to treat metastatic cancer. These results highlight a role for Eo in preventing tumor cell colonization to metastatic sites and suggest that developing drugs to trigger Eo degranulation may serve as a viable therapeutic option to treat metastatic disease.

Supervisor: Dr. Kevin Bennewith

Rachel A Cederberg[1,2], S. Elizabeth Franks[1], Brennan J Wadsworth[1,2], Michael R Hughes[3], Kelly M McNagny[3], Kevin L Bennewith[1,2]

Affiliations:
[1]Integrative Oncology, BC Cancer, Vancouver, BC. [2]Department of Pathology and Laboratory Medicine, UBC. [3]Deparment of Medical Genetics, UBC

Eric Hempel

GRADUATE STUDENT

ABSTRACT #19

Supervisor: Drs. Linda Hoang; Meghan McLennan

Eric Hempel[1], Meghan McLennan[2], Hind Sbihi[3,4], Paul Levett[3,5] , Linda Hoang[3,5]

Affiliations:
[1]Talent on Demand Program, Provincial Health Services Authority. [2]Provincial Laboratory Medicine Services, Provincial Health Services Authority. [3]British Columbia Centre for Disease Control Public Health Laboratory. [4]School of Population and Public Health, University of British Columbia. [5]Department of Pathology and Laboratory Medicine UBC

CLINICAL EVALUATION OF THE BD VERITOR™ AND NASAL ABBOTT PANBIO™ COVID-19 RAPID TESTS FOR THE DETECTION OF SARS-COV-2 AMONG SYMPTOMATIC INDIVIDUALS WITH POTENTIAL SARS-COV-2 INFECTION

Background/objectives: Health Canada recently approved two antigen-based immunoassays, the BD Veritor™ and the nasal Abbott Panbio™ rapid antigen tests (RATs), to detect SARS-CoV-2 in individuals experiencing symptoms consistent with COVID-19. Despite Federal approval, provincial studies are required to verify the manufacturers' claimed performance characteristics in a local setting. The aim of this validation was to assess the real-world performance of the Veritor™ and nasal Panbio™ in a high-prevalence population to provide further guidance for Public Health decision-making.

Methods: Two clinical validations were conducted at a COVID-19 collection Centre in Surrey, the first involving the Veritor™, followed by the nasal Panbio™ RAT. Symptomatic individuals (≥18 years of age) were asked to participate in these validations. Parallel testing was carried out on consenting individuals by standard of care (SOC) using either a nasopharyngeal swab collection or saline gargle and by RAT. SOC samples were tested within 48 hours after collection at one of the nearby COVID-19 testing laboratories with a lab-developed test using reverse-transcriptase polymerase chain reaction (RT-PCR). RAT samples were tested immediately onsite. Sensitivity and specificity were the primary outcomes of both validations to compare the performance of the RATs against the gold-standard RT-PCR.

Results: In total, 442 individuals (N=442) participated in the validation of the Veritor™. The overall sensitivity and specificity were 61.5% [95% confidence interval [CI] = 50.4% - 71.6%] and 99.5% [98.0% - 99.8%], respectively. Two hundred and three individuals (N=203) participated in the nasal Panbio™ validation. The overall sensitivity was 65.3% [51.3% - 77.1%] and the specificity was 100% [97.6% - 100%]. The cycle threshold (Ct) values of SARS-CoV-2 positive SOC specimen were stratified into several cut-off ranges to characterize the risk of transmission of affected individuals and matched with the corresponding RAT results. When the cut-off was set to only include SOC specimens with Ct values below <25, which corresponds to highly infectious individuals, the sensitivity of both the Veritor™ and nasal Panbio™ increased to 86.1% and 82.4%, respectively.

Conclusions: The overall sensitivity of the Veritor™ and nasal Panbio™ RATs is poor compared to RT-PCR, especially in detecting infection in individuals with lower viral loads. However, both RATs detect cases with lower Ct values, which correspond to individuals shedding high viral loads and who have an increased risk of transmission. Coupled with their ease-of-use and rapid turnaround time, the Veritor™ and nasal Panbio™ could be another valuable tool to quickly identify individuals with the highest risk of transmission and limit the spread of COVID-19.

Michael Steel

RESIDENT

COLORECTAL ADENOCARCINOMAS DIAGNOSED FOLLOWING A NEGATIVE FAECAL IMMUNOCHEMICAL TEST SHOW HIGH-RISK PATHOLOGICAL FEATURES IN A COLON SCREENING PROGRAMME

ABSTRACT #20

Background/objectives: The faecal immunochemical test (FIT) is used every 2 years to screen average-risk British Columbians aged 50–74 years, with follow-up colonoscopy for positive results. Non-screen-detected colorectal adenocarcinomas are defined as those detected within 25 months following a negative FIT. We aimed to more clearly characterize these malignancies.

Methods: A medical chart and focused pathology review of colorectal malignancies from 926 individuals who completed FIT in the British Columbia Colon Screening Program in 2014, and whose pathology reports were available for review, was conducted. This cohort was divided into two groups: individuals with colorectal adenocarcinomas diagnosed following a positive FIT (screen-detected) and individuals with colorectal adenocarcinoma diagnosed within 25 months of a negative FIT (FIT-interval cancers). Rates of clinically relevant pathological parameters, as outlined in the American Joint Committee on Cancer (AJCC), 8th edition, were compared between the screen-detected and FIT-interval cancer groups.

Results: A total of 876 screen-detected and 50 FIT-interval cancers were identified. FIT-interval cancers exhibited higher rates of high-grade differentiation (including poorly differentiated and undifferentiated cases; $P < 0.01$) and aggressive histotype (signet ring cell and mucinous carcinomas; $P < 0.01$) than did screen-detected cancers after Bonferroni correction. Colorectal adenocarcinoma diagnosed after a negative FIT may therefore be associated with worse prognostic determinants than screen-detected cancers.

Conclusions: FIT-interval cancers are associated with high-risk pathological features; the possibility that more aggressive, fast-growing lesions which arise in the interval after truly negative FITs cannot be ruled out. Further study of a larger cohort of FIT-interval cancers controlling for interaction among the different pathologic parameters will be undertaken.

Supervisor: Dr. David Schaeffer

Michael J. Steel[1,2], Hussam Bukhari[1,2], Laura Gentile[3], Jennifer Telford[3,4] & David F. Schaeffer[1,2,3]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, Vancouver General Hospital. [2]Department of Pathology and Laboratory Medicine, UBC, [3]British Columbia Colon Screening Program, and [4]Division of Gastroenterology, St Paul's Hospital, UBC

Jenny Chu

RESIDENT

ABSTRACT #21

Supervisor: Dr. David Schaeffer

Jenny E Chu[1], Benny Johnson[2], Laveniya Kugathasan[3], Van K Morris[2], Kanwal Raghav[2], Lucas Swanson[3], Howard J Lim[3], Daniel J Renouf[3], Sharlene Gill[3], Robert Wolber[1], Aly Karsan[1,3], Scott Kopetz[2], David F Schaeffer[1], Jonathan M Loree[3]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, Vancouver Coastal Health Authority. [2]The University of Texas MD Anderson Cancer Center, Houston, Texas. [3]BC Cancer Agency

POPULATION-BASED SCREENING FOR BRAF V600E IN METASTATIC COLORECTAL CANCER REVEALS INCREASED PREVALENCE AND POOR PROGNOSIS

Background/objectives: *BRAF*^{V600E} mutations portend poor prognosis in metastatic colorectal cancer (mCRC); however, the true prevalence and prognosis are unknown, as unwell patients may not undergo *BRAF* sequencing.

Methods: We reviewed a population-based cohort of 1,898 patients with colorectal cancer that underwent reflexive IHC mismatch repair (MMR) and *BRAF*^{V600E} testing. Outcomes among IHC-detected *BRAF*^{V600E} mCRC (*BRAF*$_{IHC}$) were compared with patients with next-generation sequencing (NGS)-identified *BRAF*^{V600E}-mutated mCRC from two institutions (*BRAF*$_{NGS}$) with patients spanning from 2004 to 2018.

Results: All-stage population prevalence of *BRAF*^{V600E} was 12.5% (238/1,898) and did not differ between early and metastatic stages (P = 0.094). Prevalence among mCRC was 10.6% (61/575), of whom 51 (83.6%) were referred to oncology and 26 (42.6%) had NGS testing. *BRAF*$_{IHC}$ had worse median overall survival (mOS) than *BRAF*$_{NGS}$ [5.5 vs. 20.4 months; HR, 2.90; 95% confidence interval (CI), 1.89-4.45; P < 0.0001], which persisted in multivariate analysis (P < 0.0001). Across a combined NGS and IHC cohort, *BRAF*^{V600E} tumors with deficient MMR showed worse mOS compared with MMR proficient tumors (8.9 vs. 17.2 months; HR, 1.46; 95% CI, 0.96-2.27; P = 0.043). In this combined cohort, first-line progression-free survival was 5.9 months, with minimal differences between regimens. Within the population-based cohort, attrition between treatment lines was high with only 60.7% receiving first-line chemotherapy and 26.2% receiving second line.

Conclusions: Patients with *BRAF*^{V600E}-mutated mCRC have a worse prognosis than previously suggested, potentially arising from referral bias for testing. High attrition between lines of therapy suggests efficacious therapies need to be prioritized early for patients to benefit.

Lisa Borretta

RESIDENT

DO TERT PROMOTER MUTATIONS CORRELATE WITH AGGRESSIVE BEHAVIOUR IN AMBIGUOUS MELANOCYTIC LESIONS?

ABSTRACT #22

Background/objectives: The majority of melanomas can be distinguished from melanocytic nevi histologically, however there are a subset of lesions that are difficult to classify that are known as ambiguous melanocytic proliferations (AMPs). An adverse outcome (recurrence, regional or systemic metastasis) occurs in 1-4% of patients with AMPs. TERT promoter mutations are reported in up to 65% of primary cutaneous melanomas and are rare in melanocytic nevi. Four hotspot mutations that create new transcription factor binding sites predominate. Recent studies suggest that TERT promoter mutation status distinguishes melanoma from benign nevi with an accuracy approaching the current melanoma FISH assay, however the predictive value in AMPs has not yet been established. We aim to determine whether TERT promoter mutations correlate with aggressive behaviour in AMPs.

Methods: Cases meeting the inclusion criteria of an AMP with >5 years of clinical follow-up are actively being identified by database search that includes hospitals across British Columbia. Histologic diagnosis and presence of sufficient residual tumor tissue are confirmed prior to DNA extraction and mutational analysis. Associations between mutation status and clinicopathologic features will be determined by statistical analysis.

Results: Ongoing chart review has thus far returned nine cases of AMPs with an adverse outcome. DNA was extracted from seven cases thus far and yielded sufficient material for molecular analysis. A TERT promoter hotspot mutations was detected in four of the cases tested thus far (57%).

Conclusions: Overall, these studies will help to determine the potential of TERT promoter mutation testing as a rapid and cost effective molecular tool for the diagnosis of ambiguous and unpredictable melanocytic proliferations.

Supervisor: Dr. Basil Horst

Lisa Borretta[1], Basile Tessier-Cloutier[1], Basil A. Horst[1]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

Kevin Shopsowitz

RESIDENT

OPTIMIZED RADAR-PLOTS FOR B-ALL MINIMAL RESIDUAL DISEASE ANALYSIS

ABSTRACT #23

Supervisor: Dr. Suzanne Vercauteren

Kevin E. Shopsowitz[1], Lorraine Liu[2], Audi Setiadi[1,2], Suzanne Vercauteren[1,2]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC. [2]Division of Hematopathology, British Columbia Children's Hospital

Background/objectives: Flow cytometry is a widely used method for B-ALL MRD analysis given its speed, availability, and sensitivity; however, distinguishing B-lymphoblasts from regenerative B-cells is not always straightforward. Radar-plots, which facilitate data visualization by projecting multiple markers onto a single plot, have been recently applied to other MRD analyses. Here we aimed to develop optimized radar plots for B-ALL MRD analysis.

Methods: Post-induction Children's Oncology Group (COG) B-ALL MRD flow data were compiled from 17 MRD-positive and 9 MRD-negative cases (enriched for hematogones). Samples were split into training and validation sets with gated CD19-positive events extracted and labeled as blasts/hematogones/mature B-cells. Radar-plot libraries for tubes 1 and 2 of the COG panel were randomly generated de novo or by perturbing manual radar-plots, and rank-ordered based on the performance of learned support vector machine (SVM) models at distinguishing normal (i.e., mature B-cell or hematogone) from abnormal (i.e., blast) events in the training data. Top-performing plots were further optimized via additional rounds of perturbation/ranking/selection, tested with the validation data, and integrated into a clinical workflow.

Results: SVM area under the ROC curve (AUROC) for COG tube-1/tube-2 radar-plots improved from 0.949/0.921 to 0.989/0.968 after optimization. This performance was maintained with the validation data and was better than for principal component analysis plots (tube-1/tube-2 AUROC = 0.931/0.850). When integrated into an MRD workflow, optimized radar-plots enabled visualization and gating of B-lymphoblasts along with different stages of B-cell maturation.

Conclusions: Radar-plots were successfully optimized to discriminate between diverse B-lymphoblast populations and maturing B-cells in B-ALL MRD analysis. Our novel radar-plot optimization strategy could be adapted to other MRD panels and clinical scenarios.

Taylor Salisbury

RESIDENT

HISTOLOGICAL SUBTYPE IS ASSOCIATED WITH PD-L1 EXPRESSION AND CD8+ T-CELL INFILTRATES IN TRIPLE-NEGATIVE BREAST CARCINOMA

ABSTRACT #24

Background/objectives: Assessment of programmed death-ligand 1 (PD-L1) expression and CD8+ tumor-associated lymphocyte levels in triple-negative breast carcinoma (TNBC) can provide valuable prognostic and predictive information. Immune-checkpoint inhibitors targeting PD-L1 have shown a survival benefit in TNBC patients with PD-L1-positive tumors in some clinical trials. However, significant variability in PD-L1 testing and interpretation has created challenges in implementing reproducible, standardized methods for accurately selecting for patients appropriate for treatment with immunotherapy. Knowledge of clinical and pathological factors that impact PD-L1 assessment are urgently needed.

Objectives: We aim to assess for an association between histological subtypes of TNBC and tumor microenvironment type, as defined by each tumor's PD-L1 status and the presence of CD8+ tumor-associated lymphocytes. We also examine the relationship between progression-free survival (PFS) and tumor microenvironment and assess for an association between PD-L1 expression and the presence of CD8+ T-cell infiltrates.

Methods: Tissue microarrays consisting of 72 TNBC cases (28 conventional invasive ductal carcinomas (IDCs), 21 basal-like IDCs, 18 apocrine carcinomas, and 5 metaplastic carcinomas) were evaluated for PD-L1 expression using the SP142 and 22C3 immunohistochemical (IHC) assays. PD-L1 positivity was defined as immune cell (IC) staining ≥ 1% (with SP142) or Combined Positive Score (CPS) ≥ 1% (with 22C3). CPS was determined according to the sum of immunoreactive tumor cells and immune cells, divided by the total viable tumor cells, and multiplied by 100. The percentage of tumor-associated lymphocytes expressing CD8 was calculated using QuPath (open-source software platform) on CD8 IHC-stained digital slides of the TMA. A CD8+ T-cell percentage of ≥ 5% was considered CD8+. Cases were separated into four tumor microenvironment groups (PD-L1+/CD8+, PD-L1+/CD8-, PD-L1-/CD8+, and PD-L1-/CD8).

Results: Basal-like IDCs were more often PD-L1-/CD8- (71.4%/61.9% of cases with SP142/22C3, respectively), while conventional IDCs showed greater variability in their tumor microenvironments (35.7% PD-L1+/CD8+ and 42.9% PD-L1-/CD8- with 22C3). All apocrine carcinomas were PD-L1-negative and tended to have PD-L1-/CD8- microenvironments (83.3% of cases with both assays). Metaplastic carcinomas were PD-L1-/CD8- in 60% of cases with both 22C3 and SP142. A CD8+ T-cell percentage of ≥ 5% strongly predicted PD-L1 positivity (positive predictive value using the 22C3 assay: 0.75). A log rank test demonstrated no significant difference in PFS among tumor microenvironment groups.

Conclusions: Our data suggest that some histological subtypes of TNBC, particularly apocrine carcinoma and conventional IDC, may help predict PD-L1 status and CD8+ T-cell infiltrate levels. In addition, the strong correlation between increased CD8+ T-cell infiltrates and PD-L1 positivity suggests that assessment of CD8+ T-cell infiltrates may serve as an alternate or compliment biomarker to PD-L1 testing to improve our ability to select for TNBC patients likely to benefit from immunotherapy.

Supervisor: Dr. Gang Wang

Taylor Salisbury[1], Alisa Abozina, Ciro Zhang, Elaine Mao, Norbert Banyi, Joyce Leo[2], Diana Ionescu[3], Chen Zhou[3], Gang Wang[3]

Affiliations:
[1]Anatomical Pathology Residency Program, UBC. [2]Department of Pathology, Royal Jubilee Hospital. [3]Department of Pathology, BC Cancer Agency

Hussam Bukhari

RESIDENT

MOLECULAR CONFIRMATION OF ALPHA 1-ANTITRYPSIN DEFICIENCY IN LIVER TRANSPLANT SETTING: A PROVINCE-WIDE EXPERIENCE

ABSTRACT #25

Supervisor: Dr. HuiMin Yang

Hussam Bukhari[1], Andre Mattman[2], Gordon Ritchie[2], Laura Burns[2], David Schaeffer[1], HuiMin Yang[1]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, Vancouver General Hospital, UBC. [2]Department of Pathology and Laboratory Medicine, Providence Health Care, UBC

Background/objectives: Alpha 1-Antitrypsin (A1AT) deficiency is a hereditary autosomal recessive disorder which affects A1AT, the main plasma protease inhibitor, leading to an increased risk of pulmonary and liver disease including the risk of developing hepatocellular carcinoma. The most affected A1AT alleles are the Z and S alleles. Patients suspected of harboring an A1AT abnormality based on lower serum concentration of the enzyme are confirmed via serum isoelectric focusing and/or PCR based DNA tests using peripheral blood samples. Determining A1AT genotype via molecular analysis of Formalin-Fixed Paraffin-Embedded (FFPE) tissue specimens is a novel approach that could aid in the detection of abnormal A1AT alleles.

In this study, we performed real-time PCR assay of FFPE liver explant tissue in specimens that showed PAS-positive, diastase-resistant (PASD) and A1AT immunoreactive globules to estimate the frequency of A1AT deficiency in liver transplant patients in the province of British Columbia.

Design: A total of 142 patients with end-stage liver disease had received liver transplants in British Columbia, Canada from May 1, 2016 to May 1, 2019. Of those patients, 18 (12.68%) showed periportal globules positive for PASD and A1AT immunohistochemical stain.

FFPE tissue blocks from these 18 transplant hepatectomy specimens were selected for real-time PCR analysis to detect S and Z allele variants. Gene sequencing of rare alleles variants is to be performed if the PCR result is negative. Additionally, a second age and sex matched control group consisting of 5 patients who also received liver transplants during the same period but who did not demonstrate PASD positive globules were included.

Of the 18 patients tested, 7 patients (38.9%) had normal pre-transplant A1AT serum levels, 3 patients (16.7%) had a low serum A1AT levels and 8 patients (44.4%) were not tested preoperatively for A1AT serum concentration. Real-time PCR assay of the FFPE tissue was successful with all the tissue samples meet quality control parameters. All the patients included in the study elucidated Z allele variants; 2 of which are homozygous (11.1%) and 16 heterozygous (88.9%). All the cases in the control group demonstrated normal wild-type MM allele.

Conclusions: Screening for Alpha 1-Antitrypsin deficiency using serum A1AT levels is not sufficiently sensitive to detect A1AT deficiency in British Columbia, especially in carriers of heterozygous alleles, leading to under diagnosis of this condition. If A1AT testing was not performed preoperatively and the risk is high based on the histological presence of PASD and A1AT positive globules in the explanted liver, then molecular testing of FFPE tissue can be a viable method for confirming the diagnosis of A1AT deficiency. In addition, we advocate for the routine use and careful examination of the PASD special stain in explanted livers to detect A1AT globules with subsequent confirmation by A1AT immunohistochemistry, even in cases with negative PASD in the preoperative biopsies.

Kimberly Hamilton

RESIDENT

CHRONIC MENINGITIS WITH LYMPHOID FOLLICLE-LIKE STRUCTURES ASSOCIATED WITH COCAINE-INDUCED MIDLINE DESTRUCTIVE LESION, A CASE REPORT

ABSTRACT #26

Background/objectives: The presence of lymphoid follicles in the leptomeninges is a very rarely seen histological pattern, previously only described in low-grade lymphomas involving the meninges and in multiple sclerosis. We report for the first time the pattern of chronic meningitis with lymphoid follicle-like structures in the context of Cocaine-Induced Midline Destructive Lesion (CIMDL). We review the pathology and clinical and radiological findings and discuss a review of the literature.

Supervisor: Dr. Veronica Hirsch-Reinshage

Kimberly Hamilton[1], Veronica Hirsch-Reinshagen[2]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Pathology and Laboratory Medicine, UBC

Tessa Bendyshe-Walton

UNDERGRADUATE STUDENT

USE OF ANTI-MULLERIAN HORMONE TESTING IN PEDIATRICS

ABSTRACT #27

Supervisor: Dr. Vilte Barakauskas

Tessa Bendyshe-Walton[1], Vilte Barakauskas[2,3]

Affiliations:
[1]UBC Faculty of Medicine. [2]Dept of Pathology and Laboratory Medicine, UBC. [3]Dept of Pathology and Laboratory Medicine, BC Children's and Women's Hospital

Background/objectives: Anti-Mullerian Hormone (AMH) testing has consistently demonstrated strong clinical utility in assessing ovarian reserve with more accuracy than serum follicle-stimulating hormone (FSH) alone. In addition to its well-established use in fertility assessment, AMH has been recognized as a useful biomarker in pediatric disorders of sexual development, gonadal axis disorders, childhood polycystic ovarian syndrome (PCOS), patients with granulosa cell or sex cord stromal tumours, and in the assessment of ovarian reserve following cancer treatment with gonadotoxic therapies. Despite its wide range of reported uses, AMH testing in pediatrics has been limited. Additionally, limited pediatric normative reference interval data exist in this patient population making interpretation of the lab values difficult. The objectives of this study were to determine current uses, barriers, and facilitators of ordering AMH testing at BC Children's and Women's Hospital (BCCWH).

Methods: In order to determine the current uses of AMH testing, a retrospective chart review of all patients who have had AMH testing ordered over the past 4 years was conducted. Semi-structured interviews were conducted with pediatric endocrinologists, oncologists and gynecologists at BCCWH to assess provider knowledge, comfort interpreting AMH results, familiarity and experience with testing, and to identify barriers to its use.

Results: Between July 2016 and March 2020, AMH testing was ordered for 47 patients with the main indication being for ovarian reserve assessment following chemotherapy or radiation (81%). Of those assessed for ovarian reserve, 47% had an AMH value below their age-specific reference interval and 38% of patients had their management changed based on their AMH results. Semi-structured interviews were conducted with 11 clinicians, 7 of which had ordered AMH testing before. Conveyed indications for testing were consistent with what was determined in the chart review and included ovarian reserve, turner syndrome, presence of functional testicular tissue, persistent Mullerian duct syndrome, pubertal delay, ambiguous genitalia and others. Common themes identified regarding current available evidence for AMH testing included: the unique benefits and information provided by AMH, concerns around age-specific reference interval data, and the need for additional evidence for its use. In terms of barriers towards ordering AMH testing, common themes that emerged included: challenges and administrative burden of ordering AMH testing, lack of confidence around the evidence and interpretation of AMH, and limitations around female fertility preservation in this patient population.

Conclusions: AMH testing has emerged as a potentially valuable tool in pediatric reproductive and endocrine disorders and in patients who have received gonadotoxic therapies. Despite this evidence, its use remains limited due to a number of barriers identified in this study. Additional studies expanding and standardizing pediatric reference interval data on newer assay platforms will aid in providing appropriate AMH values for use in clinical decision-making.

Damian Feldman-Kiss

UNDERGRADUATE STUDENT

INTERFERENCE ASSESSMENT OF KETONE BODIES ON LABORATORY PEDIATRIC CREATININE MEASUREMENT: A MULTI-CENTRE STUDY

ABSTRACT #28

Background/objectives: Recent reports have indicated that a high proportion of children hospitalized for DKA developed acute kidney injury (AKI), highlighting the importance of accurate diagnosis and follow-up testing. The diagnosis and staging of AKI involve measuring serum creatinine (Cr). However, the presence of ketone bodies (KB) that are characteristic of DKA, including acetone (Ac), acetoacetate (AA), and beta-hydroxybutyrate (BOHB) have been reported to interfere with Cr measurement in both enzymatic and Jaffe methods leading to falsely high and falsely low results, with less interference observed in enzymatic methods. Although many pediatric hospitals use enzymatic methods, general hospitals tend to rely on Jaffe methods due to their lower costs. This interference presents a particular challenge for clinicians as accurate Cr measurement is necessary to diagnose, stage, and manage AKI. To investigate the potential KB interference profiles in our region, we undertook a multi-centre study to assess the accuracy of two enzymatic and two Jaffe methods in comparison to the gold standard, liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Residual patient plasma samples were pooled to approximately 50, 100, and 250 uM Cr and spiked to final concentrations of acetone (0 to 50 mM), acetoacetate (0 to 20 mM), or BOHB (0 to 20 mM). Aliquots were distributed for testing by either one of two enzymatic assays (Ortho VITROS 5600 (E1) or Roche cobas C501 (E2)), or one of two Jaffe methods (Beckman Coulter UniCel DxC 800 (J1) or Siemens VISTA 1500 (J2)), or LC-MS/MS in duplicate. The results were analyzed with Microsoft Excel and GraphPad Prism. The % difference in Cr was calculated for each method with each KB. Interference was defined as exceeding +/-15%.

Results: E1 and E2 were largely unaffected by the presence of KBs as the % difference relative to Cr levels by LC-MS/MS for treatment groups were <15% (range, -12% to 8%). Similarly, J1 and J2 were largely unaffected by the presence of BOHB (range, -1% to -10%). The presence of Ac resulted in highly significant (P value range, P<.01 to P<.0001) dose-dependent positive interference in both Jaffe methods, whereas the presence of AA resulted in highly significant (P value range, P<.01 to P<.0001) dose-dependent positive and negative interference in J1 and J2, respectively. The absolute magnitude of interference by Ac and AA in J1 and J2 was inversely proportional to the Cr concentration.

Conclusions: Compared to the enzymatic methods, the Jaffe methods tested here were much more susceptible to interference by Ac and AA, especially at lower Cr values, which are commonly seen in pediatrics. Given this, when these Jaffe methods are used to measure Cr in children with DKA, true renal function can be unclear, which complicates the diagnosis and management of AKI if these methods are used without awareness of method-specific biases. Therefore, understanding the KB interference profiles of different Cr methods is crucial for the improvement of DKA patient care. One potential quality improvement intervention is to standardize all of the Cr methods in our region to an enzymatic method.

Supervisor: Dr. Li Wang

Damian Feldman-Kiss[1,2], Dailin Li[2,3], Richard Cleve[2,4], Graham Sinclair[2,5,6], Joshua A. Dubland[2,5,6], Li Wang[2,5,6]

Affiliations:
[1]Island Medical Program, Faculty of Medicine, UBC, Victoria. [2]Department of Pathology and Laboratory Medicine, Faculty of Medicine, UBC. [3]Vancouver General Hospital. [4]Surrey Memorial Hospital. [5]BC Children's Hospital. [6]BC Children's Hospital Research Institute

Steven Seong Gu Park

UNDERGRADUATE STUDENT

ABSTRACT #29

Supervisor: Dr. Christopher Lowe

Steven Seong Gu Park, Nancy Matic, Jennifer Bilawka, Leah Gowland, Willson Jang, Christopher Lowe

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Pathology and Laboratory Medicine, St.Paul's Hospital, Providence Health Care

IDENTIFYING GAPS IN THE APPROPRIATENESS OF BLOOD CULTURE COLLECTION VOLUMES WITHIN TWO ACUTE CARE HOSPITALS

Background/objectives: Blood cultures are one of the most important specimens handled in Medical Microbiology, and an important quality indicator to measure is the volume of blood collected in each bottle. The objective of the study was to assess pre-analytical factors that may affect the collection of optimal blood culture volumes, such as collection location or time, which may contribute to inadequate blood culture collection volumes at St. Paul's Hospital (SPH) and Mount Saint Joseph Hospital (MSJ).

Methods: All blood cultures collected at SPH and MSJ between August 2016 to December 2019 were reviewed. Blood cultures were incubated in the BACT/ALERT®VIRTUO® (bioMérieux), and blood culture volumes were extracted from this system. Blood cultures were reviewed to determine adequate volume (8-10 mL), and then correlated with unit collected, time of collection, type of healthcare worker collecting the sample and result of blood culture (positive or negative). Data analysis was performed with R studio (R 3.6.2).

Results: A total of 102,376 blood cultures were included in the analysis, 18.3% of the blood cultures had appropriate volume measuring between 8 – 10 mL on the VIRTUOâ, while 69% were underfilled and 12.7% were overfilled. Most underfilled volumes were between 3 mL to 4 mL, and SPH Emergency Department was the most common, specifically during overnight hours (23:00 – 05:00). When stratified underfilled blood cultures by type of healthcare worker, laboratory technologists (53%) were more likely to collect an underfilled bottles as compared to nurses (42%) [$p<0.0000001$].

Conclusions: The majority of blood cultures collected at SPH and MSJ were underfilled. There is further opportunity to improve the quality of blood cultures through focusing on pre-analytical factors such as appropriateness of blood culture volumes.

Salina Kung

MEDICAL STUDENT

BARRIERS AND FACILITATORS OF PARTICIPANT RECRUITMENT AND BLOOD SAMPLE COLLECTION IN PREGNANCY REFERENCE INTERVAL STUDIES

ABSTRACT #30

Background/objectives: Pregnancy laboratory reference interval (RI) studies collect blood samples from healthy pregnant women to establish normal ranges for the interpretation of blood test results. Pregnancy and Pediatric Reference Intervals for Safe Medicine (P^2RISM) at BC Women's Hospital is a pilot RI study that is recruiting pregnant women for maternal and neonatal blood sampling postpartum and evaluating feedback on P^2RISM involvement from pregnant women, researchers, and healthcare staff. Our goal is to identify specific barriers and facilitators impacting participant recruitment and blood sample collection in the P^2RISM study.

Methods: Mixed methods study design to collect feedback on the opinions and experiences of phlebotomists, researchers and healthcare providers in the P^2RISM study. Phlebotomists completed an online survey about the challenges and support needed for sample collections. Researchers and healthcare providers participated in telephone-based semi-structured interviews. Interviews were coded using the thematic analysis approach.

Results: Five key factors impacting recruitment success emerged from the analysis of 11 interviews, including: (1) patient interest and preferences, (2) staff availability, (3) timing-related challenges of consenting patients, (4) communication with healthcare providers, and (5) recruitment strategies used. For pregnant women, research participation may not be a priority close to labour and delivery. Increasing study promotion on social media and at routine prenatal health visits were suggested as ways to engage women earlier in pregnancy. Common barriers to hospital-based recruitment were limitations on staff availability and the time available to consent eligible patients before they were transferred to birthing suites or discharged home. The main facilitator of recruitment was support from nurses in identifying eligible patients upon hospital admission. For community-based recruitment, sending reminders and providing more study information to healthcare providers may increase recruitment at healthcare clinics.

Based on survey responses from 4 phlebotomists, common challenges cited included the venipuncture itself, due to the size of babies' veins and collections in patient rooms or in the presence of other family members. Phlebotomists suggested having more staff present to support the newborn babies, scheduling sample collections at less busy times, and providing more information to families about the sample collection process.

Conclusions: Barriers to recruiting pregnant women to laboratory RI studies exist at all levels of study involvement. Strategies to improve the success of recruitment and sample collection in future RI studies should consider patient-specific barriers, staff workload, phlebotomy-related challenges, and communication of patient eligibility and study information between researchers and healthcare providers.

Supervisor: Dr. Vilte Barakauskas

Salina Kung[1], Maggie Kinshella[1], Ann Tran[1], Karla Muyzer[2], Catherine Leung[2], Michelle Dittrick[3], Ashton Ellis[3], Wee-Shian Chan[1,4], Kate Chipperfield[1,3], Vilte Barakauskas[1,3]

Affiliations:
[1]Faculty of Medicine, UBC. [2]Patient Co-Leads, Vancouver, Canada. [3]Department of Pathology and Laboratory Medicine, BC Children's and Women's Hospital. [4]Department of Medicine, BC Women's Hospital

Kitty Ziwei Sun

UNDERGRADUATE STUDENT

ABSTRACT #31

Supervisor: Dr. Vilte Barakauskas

Kitty Sun[2], Ann Tran[1], Ashton Ellis[2], Michelle Dittrick[2], Horacio Osiovich[1,3], Suzanne Vercautern[1,2], Khosrow Adeli[4], Benjamin Jung[4], Wee-Shian Chan[1,5], Vilte Barakauskas[1,2]

Affiliations:
[1]Faculty of Medicine, University of British Columbia; [2]Department of Pathology and Laboratory Medicine, [3]Division of Neonatology, BC Children's Hospital; [4]Department of Pediatric Laboratory Medicine, Hospital for Sick Children; [5]Department of Medicine, BC Women's Hospital

PERIPARTUM REFERENCE INTERVALS FOR HIGH SENSITIVITY CARDIAC TROPONIN T, N-TERMINAL PRO B-TYPE NATRIURETIC PEP-TIDE AND PLASMA LACTATE IN VANCOUVER MOTHERS

Background/objectives: Laboratory testing is a part of maternal care when delivery or health complications threaten the wellbeing of mom and/or baby. Cardiac biomarkers troponin and B-type natriuretic peptide increase with cardiac damage or volume overload. Because symptoms of pregnancy and labour can overlap with those of myocardial infarction and heart failure, and because some pregnant women may have pre-existing cardiovascular conditions, these markers are used to help identify cardiovascular events, even during pregnancy. N-terminal pro b-type Natriuretic Peptide (NT-proBNP) increases by varying degrees throughout pregnancy and rises significantly in pre-eclampsia, while troponin is reported to be higher in pregnant women with hypertension. Similarly, identification of infection during labour and delivery is important to avoid maternal and neonatal morbidity. Elevated plasma lactate can serve as an indicator of infection. Despite the necessity of these biomarkers in acute maternal care, few reference intervals (RIs) are established during pregnancy, and even less exist for the the period of labour and delivery. This study derived normal reference intervals for three acute care biomarkers: high sensitivity cardiac troponin T, NT-proBNP and lactate in healthy women near the time of labour and delivery.

Methods: Healthy mothers aged 19 to 45, with healthy, uncomplicated, singleton pregnancies, delivering at BC Women's Hospital, were recruited for participation in the PRISM study. Women who were in labour or were being admitted for scheduled C-section or induction of labour were asked to donate blood. Lithium heparin plasma was stored frozen until study recruitment was completed. A total of 233 samples were used for cardiac biomarker analysis. Specimens were then analyzed using clinical testing methods on the Ortho Vitros 5600 or Roche e601 platforms. Reference intervals were derived using previously published approaches (Adeli et al. 2017; Horowitz et al. CLSI EP28-A3c, 2010.

Results: Reference intervals for hs-cTnT, NT-proBNP and lactate were determined. Hs-cTnT did not require partitioning and the overall 99th percentile reference limit was determined to be . Partitioning between labouring and not in-labour women was required for NT-proBNP and lactate. For NT-proBNP, the upper RI limit calculated as the 97.5%tile was 192 ng/L for mothers who were in labour and 106 ng/L for mothers who were not yet in labour. Lactate 95% reference intervals were determined to be 0.83-3.38 mmol/L and 0.70-2.07 mmol/L for labouring and non-labouring groups, respectively.

Conclusions: Local peripartum reference intervals have been derived using samples from contemporary Canadian mothers. Upper limits of NT-proBNP and plasma lactate are slightly higher in labouring mothers, and are also higher than limits used for non-pregnant women, while the upper limit of hs-cTnT was lower in this cohort, suggesting that use of pregnancy-specific RIs will improve lab test interpretation in the peripartum period.

Betty Yao

UNDERGRADUATE STUDENT

ELONGATION CONTROL OF MRNA TRANSLATION DRIVES GROUP 3 MEDULLOBLASTOMA ADAPTATION TO NUTRIENT DEPRIVATION

ABSTRACT #32

Background/objectives: Medulloblastoma (MB), the most common pediatric brain tumor and leading cause of cancer related deaths, carries a 5-year mortality rate between 20-35%. MYC genetic amplification and overexpression are associated with poor life expectancy and substantial morbidity in children suffering from MB. However, the high metabolic demand induced by MYC-driven transformation sensitizes MYC-overexpressing MB to cell death under conditions of nutrient deprivation (ND). Additionally, MYC-driven transformation is known to promote mitochondrial oxidative phosphorylation (OXPHOS).

We previously reported that eukaryotic Elongation Factor Kinase 2 (eEF2K), the master regulator of mRNA translation elongation, promotes survival of MYC-overexpressing tumors under ND. This flags eEF2K as an appealing candidate for novel and targeted therapeutic approaches to MB. Interestingly, eEF2K is overexpressed in MYC-driven MB and our preliminary proteomics data highlight large-scale alterations in OXPHOS components affecting eEF2K deficient MB cells. We therefore hypothesized that eEF2K activity is required for the selective translation of mRNAs needed for efficient OXPHOS, and for the progression of MYC-driven MB.

Methods: Multiplexed enhanced Protein Dynamic Mass Spectrometry was carried out in eEF2K knockdown MYC-overexpressing D425 MB cells to identify mRNAs selectively translated upon eEF2K activation. Timecourse experiments under ND (2 and 4 hours) were conducted in eEF2K knockout (KO) D425 cells to assess the presence of electron transport chain (ETC) complexes I-IV in their native state (via BN-PAGE), as well as transcript expression of individual ETC complex components (by qPCR). The effects of eEF2K inactivation on oxygen consumption, metabolic fluxes and mitochondrial membrane potential were studied with Seahorse technology and JC1/TMRE staining. The viability of eEF2K KO D425 cells was assessed by Incucyte system. Finally, MB orthotopic xenograft mouse models were used to confirm in vitro observations.

Results: Multiple (9 out of 10 detected) components of the mitochondrial OXPHOS pathway are selectively translated upon eEF2K activation. Inactivation of eEF2K by genetic KO leads to the disassembly of ETC complexes I-IV without affecting mRNA levels of their respective components. This effect is reversed following ND, which induces the disassembly of ETC complexes in control cells but not in KO cells. Consistently, eEF2K KO MB cells display decreased mitochondrial membrane potential and 20% increased proton leak thorough the mitochondrial membrane. In addition, eEF2K inactivation results in increased D425 cell death under ND and doubles survival of MB bearing mice fed with calorie restricted diets ($p<0.05$).

Conclusions: Control of mRNA translation elongation by eEF2K is critical for mitochondrial ETC complex assembly and efficient OXPHOS in MYC-overexpressing MB, likely representing an adaptive response by which MYC-driven MB cells cope with acute metabolic stress. Future therapeutic studies will aim to combine eEF2K inhibition with caloric restriction mimetic drugs as eEF2K activity appears critical under metabolic stress conditions.

Supervisor: Dr. Alberto Delaidelli

Betty Yao, Alberto Delaidelli[1,2], Gian Luca Negri, Que Xi Wang, Yue Zhou Huang, Albert Huang, Simran Sidhu, Joyce Zhang, Andrii Vislovukh, Sofya Langman, Christopher Hughes, Gabriel Leprivier[3], Poul Sorensen[1,2]

Affiliations:
[1]Department of Molecular Oncology, British Columbia Cancer Research Centre. [2]Department of Pathology and Laboratory Medicine, UBC. [3]Clinic for Pediatric Oncology, Hematology and Clinical Immunology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany

Amirhossein Bahreyni

GRADUATE STUDENT

ABSTRACT #33

DEVELOPMENT OF COXSACKIEVIRUS TYPE B3 AS A NOVEL AND SAFE TREATMENT FOR BREAST CANCER

Supervisor: Dr. Honglin Luo

Amirhossein Bahreyni[1,2], Huitao Liu[1,3], Yasir Mohamud[1,2], Yuan Chao Xue[1,2], Chen Seng Ng[1,2], Honglin Luo[1,2]

Affiliations:
[1]Centre for Heart Lung Innovation, St. Paul's Hospital.
[2]Department of Pathology and Laboratory Medicine, UBC.
[3]Department of Experimental Medicine, UBC.

Background/objectives: Breast cancer is the most commonly diagnosed malignancy worldwide, accounting for approximately 1.7 million new cases annually and roughly 25% of all adenocarcinomas. Conventional treatments such as radiotherapy and chemotherapy fail to significantly improve overall survival of patients with triple negative breast cancer (TNBC) (defined as human estrogen receptor positive (ER-)/ progesterone receptor positive (PR-)/ human epidermal growth factor receptor 2-negative (HER2-) in light of cancer heterogeneity and rapid development of drug resistance. Oncolytic viruses are a group of genetically engineered viruses with the ability to infect and kill cancer cells while demonstrating little to no impact on healthy tissue. Among different oncolytic viruses, Coxsackievirus type B3 (CVB3) has attracted tremendous attention owing to its incredible anti-tumor activity against various cancers. However potential toxicity of CVB3 administration, particularly cardiotoxicity, restricts its clinical application. Here, CVB3 was genetically engineered through addition of miRNA targeting sequences into the viral genome to establish a safe and efficient oncolytic virus that specifically targets breast cancer.

Methods: The miRNA-CVB3 was created by insertion of miR-145, miR-143, miR-1 and miR-216 target sequences into the 5′ UTR of the CVB3 genome using the plasmid pCVB3/T7. Western blot and real time quantitative PCR (RT-qPCR) were performed to evaluate entry ability of miRNA-CVB3 into different breast cancer cell lines including human TNBC cells (MDA-MB-231 and SUM149), human ER+/PR+/HER2- cells (T-47D and MCF-7), human ER-/PR-/HER2+ cells (SK-BR-3), as well as mouse TNBC (4T1 cells). MTT assay was conducted to assess cytotoxic effect of miRNA-CVB3 in different breast cancer cell lines. Anti-tumor activity of miRNA-CVB3 and its safety were assessed in TNBC-Balb/c mice.

Results: Western blot and RT-qPCR analysis revealed that miRNA-CVB3 is able to internalize and replicate in all TNBC cell lines as well as T-47D and SK-BR-3 cell lines. Furthermore, MTT assay confirmed that miRNA-CVB3 has substantial cytotoxic effects in these cell lines. Results showed that MCF-7 is resistant to miRNA-CVB3 that is due to low expression of Coxsackievirus and Adenovirus Receptor in this cell line. Despite showing great cytotoxic effect in vitro, Animal study revealed that that intravenous injection of miRNA-CVB3 has no impact on tumor growth in in tumor-bearing Balb/c mice. However, multi-dosages of intratumoral administration of miRNA-CVB3 led to a slight delay in tumor growth in tumor-bearing Balb/c mice. H&E staining results revealed that both intravenous and intratumoral administrations of miRNA-CVB3 can cause merely mild cardiotoxicity in mice.

Conclusions: *In vitro* data suggest that miRNA-CVB3 is a potent oncolytic virus against various breast cancer cells. Furthermore, in spite of showing less toxicity compared with wild-type, developed miRNA-modified CVB3 requires further modifications to enhance its safety as well as its anti-tumor activity to be an effective oncolytic virus in immunocompetent mouse models.

Jeffrey Boschman

GRADUATE STUDENT

THE UTILITY OF COLOR NORMALIZATION FOR ARTIFICIAL INTELLIGENCE-BASED DIAGNOSIS OF HEMATOXYLIN AND EOSIN-STAINED PATHOLOGY IMAGES

ABSTRACT #34

Background/objectives: The color variation of hematoxylin and eosin (H&E)-stained tissues has presented a challenge for applications of artificial intelligence (AI) in digital pathology. Many color normalization algorithms have been developed in recent years in order to reduce the color variation between H&E images. However, previous efforts in benchmarking these algorithms have conflicting results and none have sufficiently researched the efficacy of the various color normalization methods for improving diagnostic performance of AI systems. The objectives of this study are: (1) to systematically evaluate the benefit of color normalization algorithms for AI-based classification of H&E-stained histopathology slides and (2) to provide a strategy using one or multiple color normalization methods to consistently improve diagnostic performance of AI algorithms trained and tested on data from different centers.

Methods: To investigate the utility of eight different color normalization algorithms, a series of deep learning-based histotype classification experiments were conducted on images patches of three H&E-stained whole slide image datasets of two different cancer types (ovarian and breast). The ovarian cancer datasets are of multi-center origin. For each experiment, multiple cross validation splits and reference images are utilized to yield generalizable conclusions which are lacking in previous studies.
First, we compared the color normalization methods on classifiers trained and tested on images from the same center. Then, we studied the effects of color normalization on classifier performance when the train and test data are from different centers. Lastly, we investigated if mixing color-normalized images from different methods could counteract the inherent weaknesses of the individual methods.

Results: Our results indicate that no color normalization strategy consistently improves classification performance when both train and test data are from a single center, suggesting that prior results from other papers were incidental to reference image and dataset used. However, we show that color normalization can significantly improve the classification accuracy of images from external centers (ovarian cancer: 290% increase). As well, an augmentation strategy involving mixing images from three color normalization methods reliably increases the diagnosis of pathology images from different centers.

Conclusions: We found color normalization does not always increase AI-based classification of H&E-stained histopathology images, providing the most benefit when models are trained and tested on data from different centers. Furthermore, we introduce a novel augmentation strategy by mixing color-normalized images using three easily accessible algorithms to consistently improve the diagnosis of test images from external centers, even when the individual normalization methods had varied results. We anticipate our study to be a starting point for reliably using color normalization to improve the diagnosis of data from external centers for AI-based systems in digital pathology.

Supervisor: Dr. Ali Bashashati

Jeffrey Boschman[1], Hossein Farahani[1,2], David Farnell[2,3], Steven J.M. Jones[4], David G. Huntsman[2,4], C. Blake Gilks[2,3], Ali Bashashati[1,2]

Affiliations:
[1]School of Biomedical Engineering, UBC. [2]Department of Pathology and Laboratory Medicine, UBC.
[3]Vancouver General Hospital.
[4]BC Cancer Research Center.

Loïc Caloren

GRADUATE STUDENT

CHARACTERIZING RARE MITOCHONDRIAL DNA MUTATION HOTSPOTS IN PEOPLE LIVING WITH HIV

ABSTRACT #35

Supervisor: Dr. Helene Cote

Caloren, Loïc[1,2]; Dunn, Rachel A[1,2,3]; Ziada, Adam S[1,2] ; Côté Hélène CF[1,2,3]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Centre for Blood Research, UBC
[3]Women's Health Research Institute, Vancouver, BC, Canada

Background/objectives: Mitochondrial DNA (mtDNA) is prone to elevated mutation rates due to the mitochondrial environment being highly oxidative, as it is the site oxidative phosphorylation, and the fact that mitochondrial polymerase γ is error-prone. Despite advances in sequencing technology, the study of mitochondrial genomics remains challenging due to the heterogeneity, high copy number, and high turnover rate of the 16kb circular genome. While considerable progress has been made in characterizing the role of mtDNA mutations in cases of mitochondrial dysfunction, these are often limited to high frequency mtDNA variants and few studies have aimed to identify rare mtDNA mutations. MtDNA mutations are associated with aging and age-related diseases, though it is unclear which is the driving force behind the other. The aim of this study is to investigate drivers of elevated mtDNA mutations by examining rare mtDNA variants.

Methods: We used molecular barcoding-based sequencing to interrogate a 264bp region of the mitochondrial D-loop, a highly variable, non-coding, regulatory region of mtDNA. This was done in 500 individuals and multiple tissues.

Results: We identified rare somatic mutations, present at frequencies as low as 0.05%, and 3 regions containing multiple potential mutational hotspots observed in both peripheral blood and placenta. The frequency of mutations at specific positions varied across tissues, some positions being more frequently mutated in blood compared to placenta and vice versa. By constructing a phylogeny based on the wild type consensus mtDNA sequence of each individual, we were able to uncover patterns in mutations at these variable positions, the vast majority of which are transition mutations.

Conclusions: Together, these findings suggest that specific hotspot positions may be a main contributor to the total burden of the low-level mtDNA mutations. The abundance of transition mutations indicates that polymerase γ errors may be a driving factor.

Jennifer Cooper

GRADUATE STUDENT

METHODS FOR DETERMINING THE UTILITY OF BLOOD BASED BIOMARKERS IN PROFILING RISK OF ALZHEIMER'S DISEASE

ABSTRACT #36

Background/objectives: Currently, Alzheimer's disease (AD) is definitively diagnosed at autopsy, as clinical diagnoses can be incorrect up to 30% of the time. This creates major challenges for testing new interventions and providing appropriate and early treatment. To address this challenge, a new diagnostic research framework was developed. The ATN system assesses amyloid (A), tau (T), and neurodegeneration (N) components in living people. The main idea behind this framework is that accurate diagnosis is essential for effective treatment that targets AD specific pathologies. However, the gold standard of testing involves invasive collection of cerebrospinal fluid (CSF) and expensive neuroimaging. This study aims to convert the assessment of the ATN system to a blood-based biomarker panel as a simple, cost-effective diagnostic and prognostic tool. This will be achieved by measuring plasma Aβ40 and Aβ42 (amyloid markers), p-tau-181 (tau marker), and neurofilament light (NF-L; neurodegeneration marker), as well as glial fibrillary acidic protein (GFAP) as a potential vascular marker, which the ATN system currently lacks.

Methods: Analysis of plasma Aβ40, Aβ42, p-tau-181, NF-L, and GFAP will be conducted using Quanterix's Single molecule array (Simoa) technology, using the p-tau-181 V2and Neurology 4-Plex E Assays. Plasma samples will be obtained from the following cohorts.

The Canadian Health Measures Survey (CHMS) is a representative sample of the Canadian population from 3-79 years of age. This cohort will be utilized to generate validated reference intervals for our biomarkers to be used throughout this study in order to distinguish abnormal test results.

The Canadian Consortium on Neurodegeneration in Aging's Comprehensive Assessment on Neurodegeneration is a cohort with participants including cognitively normal controls, participants with subjective or mild cognitive impairment (SCI, MCI), and a variety of dementia groups including mild AD. Results will be analyzed to create biomarker profiles of AD, AD risk (normal, SCI, MCI) as well discrimination of AD from other dementia cohorts.

The Super-Seniors cohort from the Healthy Aging study includes healthy seniors aged 85–110 years who at recruitment have never been diagnosed with any form of dementia. Results from this cohort will be analyzed as a novel strategy of how biomarker levels may predict resistance to neurodegenerative diseases.

The UBC Clinic for Alzheimer Disease and Related Disorders is BC's tertiary referral dementia clinic with a longitudinal database and biobank of blood and CSF samples, some with autopsy-confirmed results. This cohort will validate the sensitivity and specificity of our biomarkers against the gold standard diagnosis of AD by autopsy verification, and assess the utility of the novel biomarkers in tracking progression of MCI to AD.

Conclusions: This panel of blood biomarkers will create an easily accessible, cost-effective and scalable tool that will assist in understanding an individual's disease stage and match the right intervention to the right patient at the right time.

Supervisor: Dr. Cheryl Wellington

Jennifer Cooper[1], Sophie Stukas[1], Howard Chertkow[2], Roger A. Dixon[3], Ging-Yuek Robin Hsiung[4], Angela Brooks-Wilson[5,6], Khosrow Adeli[7], and Cheryl L. Wellington[1,8,9]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, Djavad Mowafaghian Centre for Brain Health, Faculty of Medicine, UBC.
[2]Baycrest Health Sciences and the Rotman Research Institute, University of Toronto.
[3]Neuroscience and Mental Health Institute and Department of Psychology (Science), University of Alberta.
[4]Department of Medicine, UBC.
[5]Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency.
[6]Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby BC, Canada
[7]Molecular Medicine, Research Institute, Hospital for Sick Children, University of Toronto
[8]International Collaboration on Repair Discoveries, Vancouver, BC, Canada
[9]School of Biomedical Engineering, UBC.

Lisa Decotret

GRADUATE STUDENT

MODELING GLIOBLASTOMA INVASION USING AN EX VIVO BRAIN SLICE INVASION ASSAY

ABSTRACT #37

Supervisor: Dr. Kevin Bennewith

Decotret, Lisa R[1,2], Rocky Shi[1,3], Monica Hsu[1,3], & Kevin L. Bennewith[1,2,3]

Affiliations:
[1]Integrative Oncology, BC Cancer, [2]Department of Pathology and Laboratory Medicine, [3]University of British Columbia.

Background/objectives: Glioblastoma multiforme (GBM) is a grade IV brain tumour that is considered incurable. The median overall survival is 15 months despite patients undergoing surgery followed by radiation and chemotherapy. This low survival rate is partially attributed to the invasion of GBM cells into surrounding healthy brain tissue, resulting in cells escaping surgical excision/radiation. Thus, GBM cell invasion represents a major obstacle for curative therapy. Brain tissue is a unique environment that contains a high proportion of astrocytes as well as proteoglycans/glycoproteins while containing low levels of fibrous proteins (laminin, fibronectin, gelatin, etc.). Moreover, GBM cells typically invade as long strands that move along white matter tracks or blood vessels. As such, typical in vitro models of cancer cell invasion that use a cocktail of synthetic ECM proteins including laminin and collagen (i.e. MatrigelTM) lack the necessary components to accurately model the unique brain microenvironment. Here, we describe an advanced model of GBM invasion using *ex vivo* murine brain slices. The development of biologically relevant models of GBM invasion is critical to further understand mechanisms of invasion and improve treatment options.

Methods: We chose to work with two highly invasion GBM cell lines: LN229 and LN18 cells. To model *in vitro* invasion, we performed a spheroid invasion assay by embedding spheroids within Matrigel. Additionally, we developed a novel *ex vivo* brain slice spheroid invasion assay to assess GBM invasion into live brain tissue. In brief, brains were harvested from 6-week old C57BL/6 mice and 300 µm brain slices were generated using a Vibratome. LN229 and LN18 GFP-tagged spheroids were implanted onto the brain slices and cultured for 5 days. GBM invasion into the brain slice was quantified using confocal microscopy.

Results: LN229 and LN18 spheroids were embedded in 0.25, 1.0, and 5.0 mg/mL Matrigel and *in vitro* spheroid invasion was monitored for 5 days. We found invasion increased with increasing concentrations of Matrigel, suggesting ECM stiffness impacts GBM invasion. Importantly, we found LN229 and LN18 spheroids typically invade as single cells when embedded in Matrigel, which is rarely observed *in vivo*. Using the ex vivo brain slice invasion assay, we found that LN229 and LN18 spheroids embedded in brain tissue formed finger-like invasive projections that were not observed *in vitro*, which is the mode of cellular invasion we would expect to see in patients. Future work involves incorporating immortalized human astrocytes, the most abundant cell type in brain tissue, into in vitro invasion assays to determine if the presence of astrocytes will induce the formation of invasive projections in vitro and better model *in vivo* invasion.

Conclusions: Our work highlights the phenotypic differences observed using *in vitro* and ex vivo models of GBM cell invasion and argues that standard models of invasion are not sufficient for modeling the infiltration of GBM cells. Utilizing physiologically relevant model systems to study GBM invasion will improve the development of more effective therapeutics for GBM patients.

Rachel Dunn

GRADUATE STUDENT

ABSTRACT #38

LOW-FREQUENCY PLACENTA MITOCHONDRIAL DNA MUTATIONS AND PREGNANCY RISK FACTORS IN WOMEN WITH HUMAN IM-MUNODEFICIENCY VIRUS

Background/objectives: Both HIV and the antiretroviral treatment for it are known to affect mitochondrial DNA (mtDNA). This may contribute to the elevated rates of preterm birth (PTB) among the ~18 million women living with HIV (WLWH) worldwide. The causes of PTB remain poorly understood, but hypoxia, oxidative stress, and impaired placenta mitochondrial function are believed to play a role. Factors such as maternal smoking and older age, both associated with risk of PTB, have also been associated with increased mtDNA mutation burden in blood cells. The objective of this pilot study was to explore associations between placenta mtDNA mutation burden and maternal HIV status, smoking, age, or PTB.

Methods: Placenta tissue specimens were obtained from two cohorts of pregnant women: Children & Women AntiRetroviral therapy and Markers of Aging (CARMA-PREG), and the Pregnancy Study. Specimens selected included 37 WLWH (13/37 PTB) and 27 HIV-negative controls (10/27 PTB). A 264bp region of the mitochondrial D-loop, a non-coding region, was sequenced using molecular barcoding to detect rare substitutions; mtDNA mutation burden was expressed as substitutions per 10kb and analyzed using Mann Whitney or Spearman's correlation test.

Results: Univariately, increased placenta mtDNA mutation burden was associated with older maternal age and with smoking throughout pregnancy, although the latter was mitigated in women who quit smoking during pregnancy. No significant associations was detected with HIV status or PTB, however, a signal toward higher placenta mtDNA mutation burden among WLWH emerged in a sub-analysis restricted to the 252/264 fully conserved positions of the 64 mtDNA sequences.

Conclusions: This study suggests that older age, smoking, and HIV may be associated with increased placenta mtDNA mutations. Although all three factors are associated with increased risk of PTB, we failed to detect an association between PTB and mtDNA mutations. A larger study is needed to confirm independent associations, including with other pregnancy complications, and to investigate possible associations with antiretroviral therapy.

Supervisor: Dr. Hélène Côté

Rachel A Dunn[1,2,3], Evelyn Mann[3], Chelsea Elwood[4], Deborah Money[3,4], Hélène CF Côté[1,2,3], and the CIHR Team on Cellular Aging and HIV Comorbidities in Women and Children (CARMA-PREG)

Affiliations:
[1]Department of Pathology & Laboratory Medicine, UBC.
[2]Centre for Blood Research, UBC.
[3]Women's Health Research Institute, UBC. [4]Department of Obstetrics and Gynaecology, UBC

Lauren Forgrave

GRADUATE STUDENT

IDENTIFICATION OF CANDIDATE BIOMARKERS FOR FRONTOTEMPORAL DEMENTIA

ABSTRACT #39

Supervisor: Dr. Mari DeMarco

Forgrave LM[1], Moon J[2], Foster L[2,3], Mackenzie IRA[1,4], and DeMarco ML[1,5]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC. [2]Centre for High-Throughput Biology, UBC. [3]Department of Biochemistry, UBC. [4]Department of Pathology and Laboratory Medicine, Vancouver General Hospital, [5]Department of Pathology and Laboratory Medicine, St. Paul's Hospital.

Background/objectives: Frontotemporal dementia (FTD) is a form of early onset dementia that leads to behavioural and language deficits. The most common pathological type of FTD is characterized by TDP-43 aggregates (FTD-TDP). It is difficult to distinguish FTD-TDP from other dementias, particularly in the early stage of the disease. While a biofluid test for FTD-TDP is highly desired by physicians, we do not yet know what markers may help specifically identify FTD-TDP. Thus, we aimed to identify proteins that differentiate FTD-TDP from controls (i.e., symptomatically related forms of dementia and neuropathologically unaffected cases).

Methods: Proteomic analysis was performed on human brain tissues from immunohistochemically confirmed cases of FTD-TDP (n=13), related dementia controls (n=10), and unaffected controls (n=3). Tissue was homogenized and fractionated by SDS-PAGE, digested, and analyzed using high-resolution mass spectrometry (HRMS). To identify proteins that differentiate the FTD-TDP group from controls, proteomic data was subjected to both unsupervised (principal component analysis) and supervised (partition) clustering. Significance was calculated using a Kruskal-Wallis test, with Bonferroni correction for multiple comparisons. Identified candidate biomarkers were then assessed for their potential relevance to disease pathology.

Results: Unsupervised clustering revealed 10/13 FTD cases could be differentiated from controls using a single protein, which was not TDP-43. This identified protein was significantly increased in the FTD-TDP group. To identify additional candidates, a supervised analysis was used. Here we identified 3 proteins with 100% specificity, 2 with 80-85% specificity, and 1 with 77% specificity in separating the FTD-TDP group from controls. The top performing biomarkers from both types of clustering analysis were proteins involved in astrogliosis, a process stimulated by the destruction of neurons.

Conclusions: This biomarker discovery research identified 7 proteins that differentiated FTD-TDP cases from both related dementias and unaffected controls with high accuracy and several with direct functional relevant to the pathological cascade in FTD. These candidates will next undergo validation in an independent and larger cohort, and subsequently investigated in human biofluids.

Raelyn Gallant

GRADUATE STUDENT

UNDERSTANDING THE ROLE OF MACROPHAGES DURING TIMES OF BETA CELL STRESS

ABSTRACT #40

Background/objectives: Canada has declared diabetes a 'health-crises' due to the unprecedent increase in individuals diagnosed with diabetes and prediabetes each year. Diabetes is characterized by the dysregulation of blood glucose. The dysregulated blood glucose is caused by several factors including misuse or a lack of the hormone insulin in an individual. Insulin is grossly made by a specific cell type called beta-cells. Beta-cells are found in a micro-organ within the pancreas known as the islets of Langerhans. The islet of Langerhans has a specific reservoir of residential islet macrophages that self-reproduce in the micro-organ. These macrophages though few, approximately 1-2 per healthy islet, have been shown to play a role in islet health, and regulation. In several mouse models the removal of macrophages results in a more profound development of diabetes including, increased fasting blood glucose and earlier development. Prior methods used to deplete macrophages in these models have removed macrophages from the entire body making it difficult to distinguish the input from islet macrophages in times of beta-cell stress. A targeted antibody depletion technique against the residential macrophages will help focus the studies on the specific role of the islet immune cells. The hypothesis for this study is diminishing the macrophage population during beta-cell stress will result in an increase of dysregulated blood glucose and a decrease in beta cell area found in the pancreas.

Methods: 8-week-old male BL/6 mice were given an injection of S981 (anti-CSF1) or IgG control (n=4). Seven days post-antibody injection, multiple low doses of STZ (5 days, 30mg/kg) or dPBS were given. Blood glucose and weight were monitored. 14 days post STZ/dPBS an oral glucose tolerance test was done. A confirmation of depletion was evaluated by flow cytometry. A second cohort of mice were given S981 or IgG control (n=14). Seven days post-antibody injection, mini pumps were implanted containing either insulin receptor antagonist S961 or dPBS (n=7) at 2mmol/g. Blood glucose and bodyweight were monitored. Also, a fast-refeed prior to and post minipump injection was performed. Islets were isolated and evaluated by histochemistry.

Results: S981 resulted in a depletion of islet residential macrophages in a beta-cell proliferative state ($\bar{x}$ = 0.017 0.01) while maintaining an exocrine pancreas population ($\bar{x}$ = 0.103 0.18). No difference was found in body weight, fasting blood glucose or glucose tolerance in the STZ treated mice. The model using mini pumps showed a significant ($p < 0.0001$) decrease in random blood glucose in the macrophage depleted mice with S961 but no change in body weight or fasting glycemia between the groups. Additionally, fasting-refeed experiments displayed no significant difference between the mice with depleted and non-depleted macrophages.

Conclusions: The initial results are the opposite of the hypothesized outcome. This observation may suggest a proliferative action from residential islet macrophages that inhibits normal insulin secretion. These results may lead to a new therapeutic direction for the prevention or treatment of diabetes.

Supervisor: Dr. Bruce Verchere

Raelyn Gallant[1], Dominika Nackiewicz[2],Galina Soukhatcheve[3], Derek Dai[4], Mitsu Komba[5], C.Bruce Verchere[6]

Affiliations:
[1]BC Children's Hospital Research Institute, Department of Surgery, UBC.
[2]BC Children's Hospital Research Institute, Department of Pathology and Laboratory Medicine, UBC.

Kevin Gonzalez

GRADUATE STUDENT

DEVELOPMENT OF AN ANTI-THROMBOTIC AND ANTI-INFLAMMATORY COATING FOR MEDICAL DEVICES

ABSTRACT #41

Supervisor: Drs. Edward Conway and Dr. Jay Kizhakkedathu

Kevin Gonzalez[1,2], Kai Yu[1], Victor Lei[1], Edward M. Conway[1,2], Jayachandran N. Kizhakkedathu[1,2,3]

Affiliations:
[1]Centre for Blood Research, UBC.
[2]Department of Pathology and Laboratory Medicine, UBC.
[3]School of Biomedical Engineering, UBC

Background/objectives: There are several blood contacting medical devices that are widely used in medicine to enhance the quality of care. These include, for example, coronary stents, vascular grafts, and mechanical heart valves. A major problem that arises from use of such devices is a lack of biocompatibility that results in activation of enzyme cascades in the blood, leading to excess clotting and inflammation. One strategy that has been employed to prevent clotting and inflammation on medical devices is the development of specialized surface coatings, inspired by natural anticoagulant systems. Our lab has recently developed an antifouling surface coating made from polydopamine and poly(N,N-dimethylacrylamide) (PDA/PDMA), to which modified proteins/molecules, such as heparin, may be covalently bound in predetermined orientations for optimal function. This project aims to utilize the PDA/PDMA surface coating to conjugate heparin, thereby developing a new and more effective anti-thrombotic and anti-inflammatory coating that may be used for a wide range of medical devices.

Methods: We have modified unfractionated heparin (UFH) with a thiol group at the terminal end (Hep-SH), allowing it to be conjugated to the PDA/PDMA coating. After chemical modification, activated partial thromboplastin time (aPTT) was used to evaluate the anticoagulant activity of Hep-SH in plasma. When Hep-SH was conjugated to the coating, characterization of the surface was determined by surface zeta potential to measure surface charge and quartz crystal microbalance with dissipation (QCM-D) was used to measure the amount of Hep-SH and antithrombin bound to the coating in real-time, as heparin functions by binding to antithrombin. Hep-SH activity on the coating was evaluated by aPTT in diluted plasma and by a microplate-based whole blood clotting assay with Hep-SH coated surfaces.

Results: We determine that Hep-SH retains its anticoagulant function in plasma and prolongs aPTT (plasma only, 39s vs. HepSH (0.01 mg/ml), 58s). When Hep-SH was conjugated onto the PDA/PDMA coating, the surface zeta potential decreased from -7.09 mV to -37.7 mV and -21.7 mV when coated with HepSH in the presence of 1M and 3M NaCl, respectively. The surface concentration of Hep-SH determined by QCM-D was found to be 450 ng/cm2 and the surface concentration of antithrombin, presumably binding to Hep-SH was 120 ng/cm2. In doing our surface functional studies with aPTT using diluted plasma and the microplate-based clotting assay, there were no differences in aPTT or clotting between our PDA/PDMA coated surfaces conjugated with or without Hep-SH.

Conclusions: Our findings suggest that our thiol-modified heparin is active in plasma and can be bound to the PDA/PDMA surface coating; however, there is limited function of the thiol-modified heparin when on the surface. One strategy to address the limited function could be to increase the surface concentration of our modified heparin.

Forouh Kalantari

GRADUATE STUDENT

ABSTRACT #42

THE FUNCTIONAL EFFECT OF ARID1A AND PIK3CA MUTATIONS IN HUMAN ENDOMETRIAL ORGANOIDS

Supervisor: Dr. David Huntsman

Forouh Kalantari[1,2], Dawn Cochrane[2], Yemin Wang[2], Kieran Campbell[2], Germain Ho[2], Winnie Yang[2], Genny Trigo-Gonzalez[2], Lien Hoang[2], Jessica McAlpine[2], DAVID HUNTSMAN[1,2]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Molecular Oncology, BC Cancer Agency

Background/objectives: The endometrium is the dynamic innermost lining layer of uterus consists of stroma and single layer of epithelial cells that go through monthly proliferation and differentiation in a reproductive age woman. Any disruption in normal endometrium processes initiates number of pathologies including endometrial hyperplasia, endometrial cancer (EC), endometriosis, and endometriosis-associated ovarian cancers including clear cell ovarian carcinoma (CCOC) and endometroid ovarian carcinoma (ENOC). ARID1A a subunit of SWI/SNF chromatin remodeling complex is particularly mutated in gynecologic cancer. This complex uses ATP to mobilize nucleosomes to modulate enhancer accessibility, and loss of ARID1A impairs this activity. Our lab has discovered inactivating mutations of ARID1A, often together with an PIK3CA activating mutation, in about 50% of CCOC that are thought to be early events in tumorigenesis for both ovarian and uterine cancer. On its own, loss of ARID1A is insufficient for tumor formation in mouse endometrium and ovary, however, when combined with PIK3CA activation, leads to the development of cancer. In this project I will use organoid culture of human endometrium to model early initiation events of cancer progression.

Methods: I derive organoid cultures from dissociated primary normal human endometrium tissue in which ARID1A is knocked down using CRISPR-Cas9, and mutant PIK3CA is introduced using lentiviral transductions. I dissociated the organoids and performed single cell RNA and ATAC sequencing using the 10X genomics platform. The transcriptomes and chromatin accessibility compared between uninfected and double mutant conditions to elucidate the mechanisms underlying oncogenic transformation.

Results: Double mutant human organoids demonstrate phenotypic differences compared to the non-transduced ones. The mutant organoids are 3 times larger than the uninfected organoids and at later passage and manifest CCOC histopathology, including hobnail cells in H&E staining. Using single cell (sc) RNA-seq, from two different experiments we have observed loss of retinol binding protein 1 (RBP1) (FDR 0, log FC -1.24 – FDR 3.51293128821229e-298, log FC -1.34). RBP1 is decreased both in double mutant organoids and organoids with loss of ARID1A alone and this effect becomes more pronounced at later passages (FDR 2.01998124002601e-72, log FC -2.8). This observation is supported by preliminary ATAC-seq data showing altered chromatin accessibility in the RBP1 promoter/enhancer region upon ARID1A loss. Also, Western blot analyses of RBP1 expression in CCOC and endometrial cancer cell lines also demonstrates this correlation.

Conclusions: RBP1 regulates retinoic acid (RA) metabolism. Our preliminary results confirm that decreased RBP1 as a consequence of ARID1A loss could possibly contribute to oncogenesis through dysregulation of the intracellular bioavailability of RA and subsequent disruption of downstream signaling pathways. It may be that RBP1 silencing is targetable in CCOCs which will be further validated in future experiments.

Emily Kamma

GRADUATE STUDENT

ABSTRACT #43

Supervisor: Dr. Jacqueline Quandt

Emily Kamma[1], Pierre Becquart[1], Robert Carruthers[2], Anthony Traboulsee[2], Irene Vavasour[3,4], Cornelia Laule[1,3,4,5], Jacqueline Quandt[1]

Affiliations:

[1]Department of Pathology and Laboratory Medicine, [2]Division of Neurology, [3]Department of Radiology, [4]International Collaboration on Repair Discoveries (ICORD), [5]Department of Physics & Astronomy. University of British Columbia, Vancouver, BC, Canada

SERUM CD5 ANTIGEN-LIKE LEVELS DISTINGUISH SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS FROM OTHER MULTIPLE SCLEROSIS SUBTYPES

Background/objectives: Over 100,000 Canadians are living with multiple sclerosis (MS), a neurodegenerative disease characterized by inflammatory demyelination and disability following axonal loss. Approximately 50% of people with MS have the progressive form where disease worsens without recovery. Compared to relapsing MS, progressive MS lacks effective treatments. Limiting progression requires characterization of the pathological processes that distinguish disease subtypes that progress from those that do not. CD5 antigen-like (CD5L) is a predominantly macrophage-secreted protein with roles in modulating inflammation, lipid metabolism, and inhibiting cell apoptosis. Previous studies found serum CD5L levels decrease with age in healthy individuals yet are elevated in inflammatory conditions including chronic infections, psoriatic arthritis, and lupus. Given that serum CD5L has not yet been studied in MS, the objective of this study is to compare serum CD5L levels in healthy controls (HC), individuals with clinically isolated syndrome (CIS), relapsing remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS), and to determine whether serum CD5L levels are associated with age, sex, disease duration (DD), or expanded disability status scale (EDSS).

Methods: The study cohort included 35 HC, 20 CIS, 33 RRMS, 30 SPMS, and 20 PPMS participants recruited by the University of British Columbia MS Clinic. Serum CD5L levels were assessed with a commercial enzyme-linked immunosorbent assay. Correlation, univariable and multivariable analyses were used to determine the relationship between CD5L levels and age, sex, DD, and EDSS.

Results: Compared to HC (median [IQR], 4.2 [2.8-6.3] µg/ml), SPMS had elevated serum CD5L (7.0 [4.6-8.5] µg/ml, p=0.0006). There were no differences between HC and RRMS (4.8 [3.5-5.8] µg/ml) or PPMS (4.3 [3.3-5.8] µg/ml), and CIS tended to have higher CD5L (5.1 [4.0-7.5] µg/ml). Notably, CD5L levels in PPMS were low compared to SPMS (p=0.020). CD5L levels tended to correlate negatively with age in HC (p=0.063), positively with age in CIS (p=0.026), but did not correlate with age in RRMS, SPMS, or PPMS. Univariable analyses (p=0.013) showed increased serum CD5L levels in CIS and MS patients was associated with longer disease duration rather than differences in age, sex, or EDSS. SPMS was associated with increased serum CD5L levels (p=0.00006) compared to HC independent of age and sex. Independent of age, sex, DD, and EDSS, both RRMS (p=0.0041) and PPMS (p=0.0082) were associated with having lower serum CD5L levels compared to SPMS.

Conclusions: Our studies suggest that CD5L titers could reflect differences underlying neurological mechanisms in SPMS compared to RRMS or PPMS. The positive association between CD5L and disease duration in SPMS points to a distinct and chronic peripheral inflammatory profile compared to other disease subtypes. Further studies in a larger independent cohort are needed to characterize the processes driving CD5L expression in MS and its potential utility as a biomarker of MS progression.

Saumadritaa Kar

GRADUATE STUDENT

GENE-ENGINEERED STEM CELL DERIVED INSULIN PRODUCING CELLS TO IMPROVE GRAFT OUTCOMES FOR ISLET TRANSPLANTATION IN TYPE 1 DIABETES

ABSTRACT #44

Background/objectives: Type 1 diabetes (T1D) is a devastating autoimmune disease that leads to lifelong insulin dependence and affects over 300,000 Canadians. Transplanting glucose-responsive, insulin-producing beta-cells can be a curative therapy; however, the lack of organ donors, need for lifelong immunosuppression, graft failure, and graft rejection hinder widespread clinical implementation. Lack of organ donors may be addressed by inducing human embryonic stem cells (hESCs) to produce mature, beta-cells, thereby presenting an unlimited source of cells for transplantation.

Islet transplant failure can be exacerbated by islet amyloid polypeptide (IAPP) aggregation, a peptide hormone co-secreted with insulin from beta-cells. Recent studies have found amyloid deposition in islets transplanted into T1D recipients – a pathological feature common in T2D individuals – which could be a contributor to graft loss and dysfunction. The GAILSST amino acid sequence in IAPP lends it the propensity to form aggregates that contribute to islet inflammation and cell death. Pramlintide is a human IAPP analogue containing proline substitutions in the amyloidogenic region, rendering it non-amyloidogenic and non-cytotoxic. We hypothesize that genetically engineered hESC-derived beta-cells expressing a soluble form of IAPP such as pramlintide, will lead to human beta-cell sources with improved survival and function following transplantation in T1D.

Methods: hESC lines, CRISPR modified and GFP tagged to produce a pramlintide analogue, along with wild-type (WT) hESC cells will be differentiated to beta-like cells suitable for transplant. We will confirm that hESC-derived, pramlintide-expressing, functional beta cells have been generated by analysis of gene expression, histology, and insulin secretion. Diabetic NSG mice will be transplanted with 2.5 million cells (WT or pramlintide), free or encapsulated. Mice will be monitored for beta-cell function and grafts harvested at 24 weeks post-transplant will be examined for gene expression and histology (beta-cell mass, proliferation, islet amyloid).

Results: hESCs differentiated into insulin+ (and therefore GFP+) cells were sorted and reaggregated to achieve islet-like clusters. Gene expression was measured by NanoString; we observed expression of markers of beta-cell maturation and function in both WT and pramlintide expressing cells similar to that of human islets, although IAPP expression was lower in both cell lines. These preliminary data suggest pramlintide expression does not impact maturation of hESCs. Future studies will demonstrate pramlintide in these cells by qPCR, ELISA, western blot, and immunostaining.

Conclusions: Should transplants producing pramlintide have improved function (better blood glucose, insulin production) and histology (more beta-cells, less amyloid), it would provide evidence that IAPP aggregation contributes to islet transplant failure, and suggest that we have created a better beta-cell for transplant in T1D. We aim to produce a clinically viable cell source for replacement therapy in T1D, with the potential to transform the lives of thousands of Canadians living with T1D.

Supervisor: Dr. Bruce Verchere

Saumadritaa Kar[1,2], Paul Orban[1,4], Shugo Sasaki[1,3,4], Heather Denroche[1,4], Francis Lynn[1,3,4], C Bruce Verchere[1,2,4]

Affiliations:
[1]BC Children's Hospital Research Institute.
[2]Department of Pathology and Laboratory Medicine, Faculty of Medicine, UBC.
[3]Department of Cellular and Physiological Sciences, Faculty of Medicine, UBC
[4]Department of Surgery, Faculty of Medicine, UBC

Theodore Lam

GRADUATE STUDENT

INVESTIGATING THE ROLE OF CYTOSOLIC CALRETICULIN IN THE PROMOTION OF ONCOGENIC SIGNALING IN ACUTE LYMPHOBLASTIC LEUKEMIA

ABSTRACT #45

Supervisor: Dr. Chinten James Lim

Shing Tat Theodore Lam[1,3], Chi-Chao Liu[3], Chinten James Lim[2,3]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Pediatrics, UBC.
[3]Michael Cuccione Childhood Cancer Research Program, BC Children's Hospital Research Institute.

Background/objectives: Acute Lymphoblastic Leukemia (ALL) is the most common pediatric cancer in Canada. It is a malignancy in the bone marrow caused by the uncontrolled growth of precursor T- or B-lymphoblasts. Even though pediatric ALL is highly treatable, a primary cause for treatment failure is acquired drug resistance to chemotherapy. Calreticulin (CALR) is a chaperone protein highly enriched in the lumen of the endoplasmic reticulum (ER). It can also be found in lower abundance as extra-ER cytosolic CALR, the function of which remains largely unknown. Prior work in our laboratory implicated a role for CALR in enhancing drug resistance of ALL blasts, as increased binding of CALR to the cytoplasmic tail of transmembrane cell surface receptors correlated with enhanced pro-survival signaling in tumour cells. This suggests that the enrichment of cytosolic CALR may potentiate CALR's role in oncogenic signaling. This study aims to determine the role of cytosolic CALR in promoting oncogenic signaling, an excess of which may enhance leukemic blasts survival and treatment resistance.

Methods: Our laboratory has generated CALR-/- and PDIA3-/- Jurkat T-ALL cells using CRISPR-Cas9, which are useful null models to assess the requirement of CALR and its localization in oncogenic signaling. PDIA3 interacts with and is a co-chaperone with CALR, and we showed that PDIA3-/- cells express CALR in the ER, but not in the cytosol. Using these loss- and gain-of-function models of total and cytosolic CALR, I assessed the cytokine-mediated JAK/STAT oncogenic pathway. Wildtype (WT), CALR-/- and PDIA3-/- cells were treated with human IFNa-2b. Cell lysates were collected and separated using gel electrophoresis. Western blot was used to detect JAK/STAT protein expression, and densitometry was performed with ImageJ to measure the relative protein phosphorylation.

Results: Compared to WT cells, CALR-/- cells exhibited decreased STAT3 phosphorylation ($n=6$, $p=0.026$ and $p<0.001$ with 1.25 and 2.5 ng/mL IFNa). Similarly, PDIA3-/- cells also exhibited reduced STAT3 phosphorylation ($n=4$, $p=0.042$ and $p=0.041$ with 1.25 and 2.5 ng/mL IFNa), suggesting that the loss of cytosolic CALR did not reconstitute STAT3 phosphorylation observed in WT cells. In contrast, JAK1 was sufficiently phosphorylated in WT, CALR-/- and PDIA3-/- cells ($n=3$), indicating loss of cytosolic CALR did not impact upon cytokine-mediated JAK1 signaling. Furthermore, preliminary data showed that STAT5 is not expressed in CALR-/- and PDIA3-/- cells ($n=2$).

Conclusions: Cytosolic CALR plays a role in mediating STAT3 signaling in Jurkat cells but does not affect JAK1 phosphorylation. As STAT3 phosphorylation occurs downstream of JAK proteins, my results implicated a role for cytosolic CALR in coupling JAK-mediated STAT3 phosphorylation. In addition, the expression of both CALR and PDIA3 is likely to be associated with STAT5 expression in Jurkat cells. Future experiments include assessing the drug resistance profiles of these cells to drugs commonly used in chemotherapies.

Madeline Lauener

GRADUATE STUDENT

CHARACTERIZATION OF REGULATORY CD56BRIGHT NATURAL KILLER CELLS ASSOCIATED WITH THE ABSENCE OF CHRONIC GRAFT-VERSUS-HOST DISEASE

ABSTRACT #46

Background/objectives: Chronic graft-versus-host-disease (cGvHD) is a major cause of morbidity and mortality after Hematopoietic Stem Cell Transplantation (HSCT). In 3 large human cohorts, we phenotypically identified increased numbers of a $CD56^{bright}$, non-cytolytic natural killer (NK) cell population associated with the absence of cGvHD. This population, consistent with previously described regulatory NK cells (NKregs), appears to be important in the induction of operational immune tolerance after HSCT (defined as no cGvHD or late acute GvHD (aGvHD)). Our objective was to characterize the $CD56^{bright}$ NK cell population in patients with no GvHD, which can define the NKreg population associated with operational immune tolerance.

Methods: Patient samples were utilized from a well characterized multi-centre pediatric cohort (Applied Biomarkers in Late Effects (ABLE)/PMTC 1202 trial). NanoString analysis of day 100 blood samples was performed from patients developing cGvHD (N = 4), late aGvHD ≥114 days after HSCT (N = 4), or with operational immune tolerance (no late aGvHD or cGvHD) (N = 4). Prior to analysis, patient samples were enriched by sterile Beckman Coulter Astrios sorting for $CD56^{bright}$ and $CD56^{dim}$ NK cells. RNA was extracted using an RNeasy mini kit, and RNA expression was evaluated by nanoString. All statistical analyses were performed using SPSS v.22 (Mann-Whitney and T-Test Paired analysis).

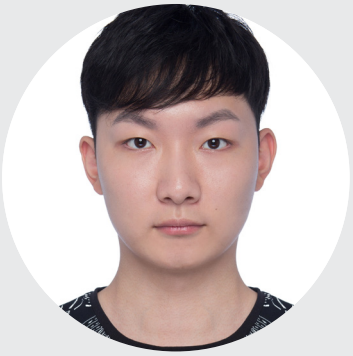
Results: We initially compared the Day 100 nanoString analysis of $CD56^{bright}$ to $CD56^{dim}$ NK cells in patients developing either a) cGvHD, b) late aGvHD or c) were tolerant with no late aGvHD or cGvHD. We found that in day 100 samples from immune tolerant patients, $CD56^{bright}$ NK cells overexpressed granzyme K (28.9-effect ratio (ER)) with no expression of perforin or granzyme B, relative to patients developing cGvHD. In addition, $CD56^{bright}$ NK cells overexpressed RNA for IL-7R (31.3-ER), RANK (18.3-ER), GM-CSFR (10.8-ER), CD62L (9.3-ER), and LEF1 (8.3-ER). Further, expression profiles of immune tolerant to late-aGvHD patients were intermediate compared to cGvHD. An evaluation of the $CD56^{bright}$ NK population associated with a loss of operational tolerance had a significant decrease in GM-CSFR expression (0.17-ER) and increase in FCGR3A/B (3.6-ER) and interferon regulatory factor 4 (3.5-ER) expression.

Conclusions: These studies show a distinct $CD56^{bright}$ NK subpopulation associated with suppression GvHD. This population expresses granzyme K and unique cytokine receptors, which are not usually associated with classic $CD56^{dim}$ NK cells. When cGvHD develops, this $CD56^{bright}$ NK population begins to display characteristics of the classic NK cell population, expressing FcRgamma and losing expression of the GM-CSF receptor. These findings may increase our future understanding of the mechanism of NKreg suppression of cGvHD, while also providing useful information for profiling and sorting for the cells, in addition to ligands to expand the population *in vitro*. Ultimately, this will contribute towards our goal of developing a NKreg adoptive transfer cell therapy for cGvHD, improving both the safety and efficacy of HSCT.

Supervisor: Dr. Kirk Schultz

Madeline Lauener[1], Amina Kariminia[1], Sayeh Abdossamadi[1], Bernard Ng[1], Shima AzadPour[1], Elena Ostroumov[1], Sara Mostafavi[2], Geoff Cuvelier[3], Kirk Schultz[1]

Affiliations:
[1]BCCHR/UBC, Vancouver, BC, Canada.
[2]University of Washington, Seattle, WA, USA.
[3]CancerCare Manitoba, Winnipeg, MB, Canada.



Zeshuo Li

GRADUATE STUDENT

INVESTIGATING THE RELATIONSHIP OF PLACENTAL MITOCHONDRIAL DNA MUTATIONS AND PRETERM BIRTH AMONG WOMEN LIVING WITH HIV

ABSTRACT #47

Supervisor: Dr. Hélène Côté

Zeshuo Li[1,2], Rachel Dunn[1,2], Hélène CF Côté[1,2], and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (Hélène CF Côté CARMA-PREG)

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

Background: Preterm birth (PTB, a birth at < 37 weeks of gestation) affects ~15 million infants annually around world and is responsible for 1 million infant deaths. Women living with HIV (WLWH) comprise approximately half of all people with HIV worldwide. In Canada, the proportion of WLWH has risen form ~5% in 1985 to ~29% in 2018. Additionally, WLWH experience a significantly higher rate of PTB (2.5-3X higher), even among women whose infection is well controlled by combination antiretroviral therapy (cART). A previous study from our lab in women found that higher blood mitochondrial DNA (mtDNA) mutation rates are significantly associated with older age and smoking. Placental mtDNA mutation between PTB and term birth (TB) from a small sample (n = 64) was studied but we did not find a significant difference. However, because of the limited sample size, a larger study will better inform this association.
Hypothesis: PTB is associated with placental mitochondrial DNA mutations.
Aims: To characterize placenta mitochondrial DNA (mtDNA) alterations as a marker of mitochondrial health/function.

Methods: I will study placenta tissue collected as part of the Children & women AntiRetroviral therapy and Markers of Aging (CARMA)-PREG cohort which enrolls pregnant WLWH (around the 13th week of gestation), and the EPIgenetics in pregnancy Complications (EPIC) study, which collects placenta tissue from women not living with HIV. Placenta DNA will be extracted from 200 WLWH treated with cART (50 PTB, 150 term births) and 200 HIV-negative women (100 PTB, 100 term births), where PTB are spontaneous or due to premature rupture of membranes (i.e. not iatrogenic). MtDNA content will be quantified by monochromatic multiplex qPCR. MtDNA deletions and heteroplasmic mutations, the latter defined as variants present at >2% of mitochondrial genomes, will be assessed by next-generation sequencing of two large (8.5-9.5kb) PCR fragments that span the entire mtDNA genome. MtDNA somatic mutation burden will be quantified using a primer-ID next-generation assay recently developed by our group. Briefly, each mtDNA molecule is labeled with a unique primer ID, and sequenced using Illumina MiSeq. Grouping of reads according to primer ID allows us to distinguish between PCR/sequencing mutations and true mutations present in the starting material.

Results: I expect to find that there is a significant difference of placental mtDNA alterations between PTB and TB, also HIV positive and HIV negative, based on the extended sample group. The deletion level of placental mtDNA is also expected to be higher in PTB group than that in TB group.

Conclusions: If I find that WLWH have more placenta mtDNA alterations than HIV-negative women, and that these alterations are a predictor of PTB, I will explore possible associations with specific antiretrovirals in pregnancy. These findings will help understand the mechanism of mitochondrial dysfunction and the role it may play in PTB, in HIV and non-HIV populations. This research may eventually contribute to predicting or preventing PTB.

Fang Fang Li

GRADUATE STUDENT

ASSAYS TO MEASURE THE IMPACT OF PREVIOUS CORONAVIRUS INFECTION ON THE IMMUNE RESPONSE GENERATED FROM SARS-COV-2 INFECTION AND VACCINATION

ABSTRACT #48

Background: The original antigenic sin (OAS) describes the immune system's preferential use of immune memory when encountering a slightly different version of a pathogen, leading to a blunted adaptive response. Therefore, previous exposures to seasonal coronaviruses (HCoV) responsible for the common cold may hinder responses to SARS-CoV-2 (SARS2) infection or vaccination, leading to worsened clinical outcomes or decreased protection due to similarities in their Spike (S) proteins. We assessed the performance of three pan-coronavirus (pan-CoV) immunoassays to determine their utility in addressing the role of OAS in COVID-19 clinical course and vaccination by validating their SARS2 diagnostic performance and comparing the detected HCoV response against one another.

Methods: 117 serum specimens were run on three pan-CoV assays. Presumed negative cases (n=86) were collected prior to the index case in Canada (January 27th, 2020) and tested negative by PCR and the BC Centre for Disease Control's serology diagnostic algorithm, while presumed positive cases (n=31) were collected following the index case and tested positive on both PCR and serology. Surface plasmon resonance imaging (SPRi) is a biosensor technology that detects antibodies against SARS2 (Receptor binding domain, RBD; nucleocapsid, NC) as well as HCoVs OC43 and HKU1 (S) through light refraction. A 96-well-based electrochemiluminescent immunoassay from Meso Scale Diagnostics (MSD) utilizes antigen spots at the bottom of a well for simultaneous detection of HCoVs OC43, HKU1, 229E, and NL63 (S), SARS-CoV (S), and SARS2 (S, RBD, NC). VirScan-CoV uses bacteriophages presenting viral antigens to capture present antibodies, leveraging the use of the entire viral proteomes of SARS2, SARS-CoV, MERS-CoV, and HCoVs OC43, HKU1, 229E, and NL63 to allow simultaneous detection of all proteins of the listed viruses. Sequencing is used to identify the exact identity of the antigens that the antibodies captured were bound to.

Results: MSD and SPRi both demonstrated high sensitivities and specificities across their SARS2 targets, with sensitivities ranging from 90.3% to 100% and specificities ranging from 91.9% to 100% across all five tests with no overlapping 95% confidence intervals. Preliminary results for VirScan-CoV (n=41; positive=3, negative=38) had a sensitivity of 100% and a specificity of 94.7% (95% CI: 81.2-98.7%). While MSD and SPRi showed consistent findings with one another in antibody titres for HCoVs HKU1 and OC43, with significant differences seen between SARS2-positive and SARS2-negative groups for each virus ($p<0.0001$), this trend has yet to be seen in preliminary results of VirScan-CoV.

Conclusions: MSD and SPRi both demonstrated high clinical sensitivity and specificity, exhibiting comparable findings in the detection of HKU1 and OC43 consistent with present literature, making them suitable for use in investigating the contribution of OAS in COVID-19 disease severity and vaccine response. VirScan-CoV has demonstrated promising potential in diagnosis thus far, but more samples must be tested before its ability to address OAS can be determined.

Supervisor: Drs. Agatha Jassem & Natalie Prystajecky

Fang Fang Li[1], Aaron Liu[2], Ebrima Gibbs[3], Guadalein Tanunliong[4], Jun Duan[1,4], Natalie Prystajecky[1,4], Mel Krajden[1,4], Muhammad Morshed[1,4], Soren Gantt[5], Neil Cashman[3], Inna Sekirov[1,4], Agatha Jassem[1,4]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Experimental Medicine, UBC.
[3]Djavad Mowafaghian Centre for Brain Health, UBC.
[4]BC Centre for Disease Control Public Health Laboratory, Vancouver.
[5]Department of Microbiology, Infectious Diseases and Immunology, University of Montréal, Montréal, QC, Canada

Michael Li

GRADUATE STUDENT

ABSTRACT #49

Supervisor: Dr. Christian Steidl

Michael Y Li[1,2], Lauren C. Chong[1], Elizabeth A Chavez[1], Bruce W Woolcock[1], Adele Telenius[1], Vivian Lam[3], Gerald Krystal[3], Elena Vigano[1], Shannon Healy[1], David W. Scott[1,2] and Christian Steidl[1,2]

Affiliations:
[1]Lymphoid Cancer Research, BCCRC.
[2]Department of Pathology and Laboratory Medicine, UBC.
[3]Terry Fox Laboratory, BC Cancer Research Centre, BCCRC.

TRAF3 LOSS-OF-FUNCTION DRIVES NON-CANONICAL NF-KB PATHWAY ACTIVATION IN DIFFUSE LARGE B-CELL LYMPHOMA

Background/objectives: Effective treatment of relapsed/refractory diffuse large B-cell lymphoma (DLBCL) remains hampered due to extensive molecular, clinical and pathological heterogeneity. To address these knowledge gaps, we set out to identify genetic drivers contributing to DLBCL disease maintenance. We report recurrent genomic deletion of tumour necrosis factor receptor-associated factor 3 (TRAF3), which encodes for a regulator of NF-kB signaling, in primary DLBCL patients uniformly treated with standard immuno-chemotherapy (7%, 22/313). RNAseq revealed a reduction of TRAF3 mRNA in deleted versus copy number neutral cases (p=0.002).

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) is a transcription factor family regulating gene expression programs such as inflammation and cell survival. NF-kB signaling occurs via canonical and non-canonical (NC) branches, and key pathway members are often somatically mutated in lymphoid cancers. We therefore hypothesize that a TRAF3 loss-of-function phenotype leads to increased NF-kB activity and cellular fitness in DLBCL.

Methods and Result: To functionally characterize the TRAF3 loss-of-function phenotype in DLBCL, we used CRISPR/Cas9 to knock out TRAF3 in four DLBCL cell lines. We performed immunoblotting of NF-kB pathway members and found increased nuclear amounts of NC NF-kB transcription factor complex subunits RelB and p52. Consistent with these findings, TRAF3 knockout cells exhibited NF-kB transcriptional upregulation by Luciferase reporter activity and elevated pro-inflammatory cytokine production (IL6, LTA) by Luminex and ELISA.

To study transcriptome changes due to TRAF3 loss-of-function, we performed gene expression profiling on our DLBCL model systems and primary DLBCL samples. We found enrichment of NF-kB associated pathways and additional enriched gene sets including cell cycle regulation and metabolism suggesting a potential proliferative and survival advantage in TRAF3-deficient DLBCL.

To assess the therapeutic targetability of the NC NF-kB pathway, we initially evaluated proteasome inhibition to block NC NF-kB activation. Surprisingly, proteasome blockade did not restore RelB and p52 nuclear localization but effectively reduced canonical NF-kB activation, indicating differential pathway regulation while utilizing a shared biochemical mechanism. Notably, we observed an accumulation of the central NF-kB-inducing kinase (NIK), a direct target of TRAF3-mediated ubiquitin-proteasome degradation, in TRAF3 knockout cells. Hence, we performed shRNA knockdown of NIK which rescued TRAF3-loss induced NC NF-kB activation. Using an NIK-specific kinase inhibitor (Isoq), we recapitulate our shRNA findings and effectively kill TRAF3-deficient cells.

Conclusions: Our findings link TRAF3 loss to clinical and gene expression phenotypes and highlight NC NF-kB activation as a patho-genetically important pathway in DLBCL. Future studies will be directed towards comprehensive evaluation of NIK inhibitors for effective blockade of constitutive NC NF-kB activation in DLBCL.

Jennifer Luu

GRADUATE STUDENT

IDENTIFYING SECRETED PROTEINS DURING MALIGNANT TRANSFORMATION OF LUNG EPITHELIAL CELLS

ABSTRACT #50

Background/objectives: Lung cancer is the leading cause of cancer-related deaths in Canada, with lung adenocarcinoma being the most common subtype. High mortality is partially attributed to late diagnosis; advanced stage lung cancer is often difficult to treat. To decrease mortality, earlier detection of the condition is necessary. Diagnostic biomarkers represent an sensitive method, one which is also relatively non-invasive, that addresses the need for earlier detection.

Biomarker development includes assessing how malignant cells can modify the surrounding microenvironment to avoid immune detection and promote growth. This can be mediated through secreted proteins. Current studies have focused on secreted protein changes during advanced stages of cancer; however, pre-neoplastic and early stages are relatively unknown. Studying changes during these stages may provide valuable information for a comprehensive, diagnostic biomarker panel. My goal is to identify secreted protein changes during malignant transformation of lung epithelial cells to lung adenocarcinoma in vitro. I hypothesize that oncogenic transformation leads to secreted proteins that alter the tumor microenvironment to promote immune evasion.

Methods: Immortalized human bronchial epithelial cells (HBEC-3KT; HBEC) were infected with lentiviral vectors expressing clinically relevant oncogenic mutations EGFR L858R or KRAS G12V, with or without expression of a p53 c-terminal construct. Cell transformation will be confirmed with anchorage-dependent and independent growth. Anchorage-dependent growth will be assayed with clonogenic and focus formation assays; anchorage-independent growth will be assessed with soft agar assay. Then, cells will be seeded and grown to confluency in 6 mililitres standard serum-free media (KSFM containing BPE and EGF). Media will be switched to one solely without serum for 24 hours; this will reduce media background and maintain cell viability. Collected media will be spun at 1000 rpm, filtered with a 0.22micrometre filter, and then concentrated with a 3kDa cut-off filter to 500 microlitres. Concentrated media will be subjected to tandem mass tag mass spectrometry. Secreted proteins that show significant relative-fold change will be validated with ELISA. Validated proteins will be assessed for immune evasion in co-culture assays.

Results: HBEC cells expressing EGFR L858R or KRAS G12V show greater clonogenic potential, relative to those with the vector control. However, cells fail to show anchorage-independent growth when grown in soft agar.

Conclusions: Oncogenic mutations EGFR L858R and KRAS G12V appear to be insufficient to confer HBEC transformation. Future work will focus on establishing a concrete, transformed phenotype in vitro to examine secreted proteins.

Supervisor: Dr. Will Lockwood

Jennifer Luu[1,2], Fraser Johnson[2,3], Will Lockwood[1,2]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Integrative Oncology, BCCRC.
[3]Interdisciplinary Oncology Program, Faculty of Graduate and Postdoctoral Studies, UBC.

Rana Minab

GRADUATE STUDENT

ABSTRACT #51

Supervisor: Drs. Bruce Verchere, Peter van den Elzen

Rana Minab[1], Sharad Purohit[2], Dong Zheng Jun[3], Mairead Cavinaw[3], Peter van den Elzen[1]

Affiliations:
[1]Department of Pathology & Laboratory Medicine, UBC.
[2]Department of Undergraduate Health Professions: Medical Laboratory, Augusta University, Augusta, GA, USA

BRAIN LIPIDS ARE RECOGNIZED BY ANTIBODIES MADE DURING INFECTIOUS MONONUCLEOSIS – ITS IMPLICATIONS FOR MULTIPLE SCLEROSIS AND OTHER AUTOIMMUNE NEUROLOGICAL DISEASES

Background/objectives: Canada has one of the highest rates of Multiple Sclerosis (MS) in the world. Virtually everyone with MS is also infected with Epstein-Barr virus (EBV). EBV, an oncogenic herpesvirus, infects B cells and since B cell depletion is a first-line treatment for MS, exploring the role of EBV-infected B cells in MS is a logical priority. Although EBV infection is largely innocuous, 75% of teens develop infectious mononucleosis (IM), which dramatically increases the risk of MS by two to three fold. IM can be diagnosed by the presence of "heterophile" antibodies which target a glycolipid antigen (Paul-Bunnell) that is like certain brain myelin markers. We hypothesize that EBV infected subjects who develop IM produce higher levels of anti-glycolipid antibodies via B cells, which cross-react with lipid components of myelin, leading to a higher risk of developing MS.

Methods: To measure antibodies against major brain glycolipids, a Luminex based array on a 96 well plate was used with fluorescent beads bound to over 200 glycolipid markers. Mean fluorescent intensity (MFI) was used to measure anti-glycolipid antibodies in patient sera. Six infant serum samples before and after EBV infection and 9 IM positive sera from young adults were tested. Negative and positive sera from an IM test kit were used as controls. All samples were run in duplicates.

Results: Results show an overall increase of anti-glycolipid antibodies against major brain lipids in IM patients but not pre-post EBV infected infants. IM positive sera had 10 times the anti-glycolipid antibodies to major brain lipids compared to pre and post-EBV infected infants ($p<0.0001$; Wilcoxon test). There was no significant difference in anti-glycolipid antibodies before and after EBV infection in infants suggesting that EBV infection alone is not leading to an increase in these antibodies but rather symptomatic development of IM due to EBV infection.

Conclusions: These results suggest that antibodies produced during symptomatic EBV infection (IM) can target brain lipids that are also degraded during MS. Future studies focused on preventing the onset of IM, or EBV infection all together, could decrease the risk of MS development greatly.

Angela Mo

GRADUATE STUDENT

ELUCIDATING THE MECHANISMS OF LEUKEMOGENESIS DRIVEN BY FBXO11 LOSS

ABSTRACT #52

Background/objectives: Acute myeloid leukemia (AML) is the most common adult leukemia, and AML patients have a 5-year survival rate of <30%. However, there has been little advancement in therapeutic options. We found frequent somatic mutations in ubiquitin proteasome system genes by exome and RNA-seq of 140 clinical AML samples, with recurrent inactivating mutations in FBXO11, which codes the substrate-recognizing component of the SKP1-CUL1-F-BOX (SCF) ubiquitin E3-ligase complex. Our mouse marrow transplant model shows that Fbxo11 knockdown cooperates with AML1-ETO expression to initiate AML. These AMLs were serially transplantable, indicating the presence of leukemic stem cells (LSC). We hypothesized that FBXO11 depletion in hematopoietic stem and progenitor cells (HSPC) promotes LSC self-renewal and drives leukemogenesis through dysregulated expression of its ubiquitination targets.

Methods: We performed quantitative tandem mass spectrometric analysis of FBXO11 co-immunoprecipitating proteins in FBXO11 CRISPR/Cas9 knockout (KO) and control clones from K562 cells to identify FBXO11-regulated targets and identified LONP1 as a top target. To determine the effects of FBXO11 and LONP1 dysregulation on hematopoesis, FBXO11 and LONP1 were over-expressed or knocked down by shRNAs with lentiviral transduction in CD34+ enriched cord blood cells. RNA was extracted by TRIzol for RNA sequencing, and cell-surface differentiation markers analyzed by flow cytometry. To determine whether FBXO11 ubiquitinates LONP1, LONP1 from the FBXO11 KO K562 cells expressing empty vector or FLAG-FBXO11 were immunoprecipitated, and blotted for ubiquitin.

Results: LONP1, a mitochondrial protease that is dysregulated in colorectal cancer, was reciprocally co-immunoprecipitated with FBXO11, but protein expression did not change with FBXO11 over-expression or loss, suggesting that LONP1 is not targeted for degradation. Knockdown of LONP1 in human CD34+ HSPCs results in similar phenotypes as FBXO11: enriched hematopoietic stem cell and LSC RNAseq signatures, increased myeloid population, and reduced erythroid and megakaryocytic populations. This suggests that FBXO11 positively regulates LONP1. We found with immunoprecipitated LONP1, that K63-linked polyubiquitination—an activating mark shown to play a role in protein trafficking—is increased with FBXO11 over-expression.

Conclusions: Our results suggest that FBXO11 positively regulates LONP1, and FBXO11 loss results in reduced LONP1 activity. We predict that this is due to reduced LONP1 localization to the mitochondria through loss of K63-linked polyubiquitination of LONP1. Our current focus is on elucidating the mechanisms of FBXO11-dependent regulation of LONP1, particularly the effects of FBXO11 loss on mitochondrial function. We have identified novel candidate targets of FBXO11, and demonstrated a unique link between the ubiquitin-proteosome system and the mitochondria. With the commonality of SCF-FBXO11 perturbations in AML, this could lead to development of new and widely applicable therapeutic options.

Supervisor: Dr. Aly Karsan

Angela Mo[1,2], Linda Chang[1,2], Gerben Duns[1], Xuan Wang[2], Elijah Willie[2], Shujun Huang[2], Derek Tam[2], Nadia Gharaee[2], Rawa Ibrahim[1,2], Rod Docking[1,2], Patricia Umlandt[1,2], Jeremy Parker[1,2], Aly Karsan[1,2]

Affiliations:
[1]Pathology and Laboratory Medicine, UBC.
[2]Centre for Clinical Genomics, Michael Smith Genome Sciences Centre, BCCA.

Kouther Noureddine

GRADUATE STUDENT

ABSTRACT #53

Supervisor: Dr. Calum MacAulay

Kouther Noureddine[1], Paul Gallagher[1], Martial Guillaud[1,3], Calum MacAulay[1,2]

Affiliations:
[1]Department of Integrative Oncology, BC Cancer Research Institute.
[2]Department of Pathology and Laboratory Medicine, UBC.
[3]Department of Statistics, UBC.

COMBINING MULTIPLEXED IMMUNO-HISTOCHEMISTRY AND DEEP LEARNING TO SPATIALLY MAP THE TUMOUR MICROENVIRONMENT

Background/objectives: The tumour microenvironment(TME) is a highly complex mixture containing epithelium, stroma and a diverse network of immune cells and the spatial organization of these immune cells within the TME reflects a crucial process in anti-tumor immunity. Not all tumour cells interact similarly; micro-environmental features are often key indicators of treatment response and patient outcome.

Methods: The usual standard of care for assessing if a patient has cancer, its stage and its likely future biological behaviour is visual examination of one or more H&E and/or Immunohistochemistry(IHC) stained sections. The paradigm of digital pathology has changed, moving from single-marker IHC towards multiplexed labeling, increasing the need for more advanced techniques that can be easily integrated in routine clinical pathology. Although recent advances in multiple immunostaining have enabled characterization of several parameters on a single tissue section. For a higher dimensional chromogen based methodology, we have developed a multiplexed IHC procedure combining multiple labels per round with multiple sequential rounds, where multiple is 12-14 chromogen based antibodies on a single tissue section. Thus, enabling analysis of complex immune cell population's on a single slide through consecutive cycles of staining, destaining, hyperspectral imaging and spectral unmixing of the chromogen biomarkers in each round. That the process presented is chromogen based (absorption microscopy) means that high throughput imaging of 12-14 markers across entire slides is feasible in a reasonable time frame.

Results: Robust, accurate, segmentation of cell nuclei for touching/overlapping nuclei is one of the most significant unsolved issues in digital pathology. Analyzing cell-cell interactions between immune and tumour cells and identifying clinically relevant patterns may improve patients outcome by informing on the likelihood of success of possible treatments. By combining a multiplexed IHC technique which enables the detection of multiple markers on a single slide with deep learning segmentation methods to recognize/segment every individual cell nuclei in tissue sections with an accuracy comparable to human annotation, we can improve the accuracy of tissue classification based upon the measured characteristics of these cells and their spatial organization.

Conclusions: These two techniques joined can be scaled up to the entire tissue section level, improving our understanding of the biological aggressiveness of specific cancers and enabling an accurate spatial cell level representation of the tissue.

Juhee Oh

GRADUATE STUDENT

NUTRIENT STRESS-INDUCED KINASE SIGNALING ENHANCES PRIMARY CD8+ T CELL FUNCTION

ABSTRACT #54

Supervisor: Dr. Ramon Klein Geltink

Juhee Oh[1,2], Annette E Patterson[1,2], Neeku Amanat[1,2], Janice Tsui[1,2], Anuli C Uzozie[1,2], Kirsten A Ward-Hartstonge[1,3], Philipp F Lange[1,2], and Ramon I Klein Geltink[1,2*]

Affiliations:
[1]BC Children's Hospital Research Institute, [2]Department of Pathology and Laboratory Medicine, [3]Surgery, University of British Columbia.

Background/objectives: CD8+ T cells are part of the immune defense against infections and cancer. CD8+ T-cell differentiation and function is strongly linked to differences in metabolic activities. Upon T-cell receptor activation, CD8+ T cells assimilate glucose (GLC) carbon mainly into glycolysis to support cytotoxic function. Previous studies showed that dampening GLC metabolism during activation or in fully activated CD8+ T effector (TE) cells confers better anti-tumour function of CD8+ T cells in vivo. This phenotype was associated with enhanced interferon (IFN)-gamma production and tumour clearance in vivo. However, the mechanism by which in vitro GLC restriction augmented in vivo function remains poorly understood. Given the limited changes in the transcriptome of GLC restricted TE cells, we focussed on posttranscriptional modifications as a result of GLC depletion. In a recent study, the stress-sensing p38 kinase was identified as a modulator of CD8+ T cell function and GLC metabolism, thus, we questioned whether nutrient-stress induced changes in p38 could be altering T cell biology.

Methods: To generate fully activated CD8+ TE cells, ovalbumin-specific murine T cells were activated for 48 h in medium containing IL-2, and chicken ovalbumin peptide, followed by 24 h of expansion. These cells were exposed to control (10mM) or limited GLC (1 mM) overnight. Losmapimod was used to determine the effects of p38 signaling. Immunoblotting was performed to confirm the inhibitory effect of p38i. Metabolic phenotypes, IFN-gamma production, and tumour killing ability were assessed by Seahorse XF bioanalyzer, flow cytometry, and tumour co-cultures respectively.

Results: Our phosphoproteomics analysis indicated that GLC restriction induced phosphorylation-mediated activation of p38. After 20-hr GLC starvation, TE cells were refed with 10 mM GLC, after which p-p38 signal was lost, implicating p38 as a GLC sensor in CD8+ TE cells. To determine the effects of the p38 signaling pathway, TE cells were treated with a p38i during GLC restriction. We confirmed that p38i treatment decreased p-p38 in a concentration-dependent manner both in control and GLC restricted TE cells. p38i treatment did not change the global metabolic changes induced by GLC restriction as assessed by oxygen consumption and extracellular acidification rate, but did blunt the augmented IFN-gamma production, and anti-tumour function, suggesting a causal role for p38 in the augmented function of in vitro GLC restriction.

Conclusions: These results suggest that the activation of p38 is regulated by GLC availability, implicating p38 as a GLC-sensing kinase in CD8+ TE cells. We are currently testing if GLC import and/or assimilation into metabolic pathways directly regulates p38 signaling. The observation that p38i dampened IFN-gamma production, suggests that p38 might connect GLC metabolism to immune function. We aim to translate these insights into the enhancement of adoptive cellular therapy of cancer.

Manideep Chowdary Pachva

GRADUATE STUDENT

DETERMINING THE ROLE OF EWING SARCOMA DERIVED EXTRACELLULAR VESICLES IN REGULATING THE TUMOR MICROENVIRONMENT

ABSTRACT #55

Supervisor: Dr. Poul Sorensen

Manideep Chowdary Pachva[1]

Affiliations:
[1]British Columbia Cancer Research Centre, UBC

Background/objectives: Ewing Sarcoma (EwS) is a highly metastatic bone and soft tissue cancer in childhood (ages 3-15). It is estimated that around 30% of the EwS patients in Canada present with metastasis at diagnosis and are at high risk with poor prognosis. Extracellular vesicles (EVs) are small membrane bound particles secreted by cells for intercellular communication by transporting cargo to recipient cells. It was recently found that EVs released from the EwS cells contain high amounts of retroelement RNAs (RE RNAs) that suppress the immune response against cancer cells through a self□propagation mechanism like viral infections, but their molecular mechanism is yet to be elucidated. We hypothesize that the RE RNAs released from EwS EVs cause RE transcription and recipient cell genetic and epigenetic reprogramming through chromatin remodelling.

Methods: To test this hypothesis, we are currently monitoring the effects of EwS□FLI1 and ADAR1 knock-down in EwS-cells, factors involved in retroelement RNAs transcription and their editing, respectively. We plan to use ATAC□sequencing and ChIP□sequencing to confirm the effects of EwS□FLI1 and ADAR1 knock□down on EwS□EV derived reprogramming of recipient cells.

Results: Our preliminary results suggest that the EVs from EwS cells cause recipient cell reprogramming and immunosuppression, only when they contain high amounts of edited retroelement RNAs.

Conclusions: This research will help us device novel strategies to block this viral-like transfer of EV-derived RE-RNAs from cancer cells to surrounding tissues, to block immune suppression and stop their metastasis.

Lindsay Pallo

GRADUATE STUDENT

DEVELOPMENT OF AN ASSAY FOR (PRO)-ISLET AMYLOID POLYPEPTIDE (1-67) IMMUNOREACTIVITY

ABSTRACT #56

Supervisor: Dr. Bruce Verchere

Lindsay Pallo[1,2], Yi-Chun Chen[1,3], Jaques Courtade[1,2], Bruce Verchere[2,3]

Affiliations:
[1]BC Children's Hospital Research Institute.
[2]Department of Pathology and Laboratory Medicine, UBC.
[3]Department of Surgery, UBC.

Background/objectives: Pancreatic beta-cells are the target of autoimmune destruction in type 1 diabetes (T1D), resulting in lifelong dependency on exogenous insulin injections. Many clinical trials are underway to improve this suboptimal therapy, requiring the availability of reliable biomarkers to evaluate beta-cell response and assess disease severity. However, common T1D peptide biomarkers are limited by their inability to be detected in subjects with long-standing disease. Islet amyloid polypeptide (IAPP) is the second most abundantly secreted peptide from beta-cells and serves as a promising candidate in the search for a more sensitive T1D biomarker. IAPP is first made as a precursor molecule, proIAPP(1-67), that is sequentially cleaved by proteolytic enzymes. Interestingly, the enzyme responsible for the first cleavage event, prohormone convertase 1/3, is reportedly decreased in T1D beta cells. We therefore hypothesize that proIAPP(1-67) is disproportionately elevated in T1D and may provide value as a disease biomarker. Here we report the development of an enzyme-linked immunosorbent assay (ELISA) with the capacity to measure circulating proIAPP(1-67) levels in T1D individuals.

Methods: Assay limit of detection (LOD) was calculated using standard curves generated by synthetic human (pro)IAPP forms. Various pairings and concentrations of monoclonal mouse antibodies (mAb) raised to different human proIAPP(1-67) epitopes were evaluated for peptide specificity. Further, incubation periods, reagent temperatures, and diluent buffers were additionally optimized. Chemiluminescent signals were detected using the MesoScale Discovery platform. Plasma matrix effects were assessed by performing spike and recovery analysis using human non-diabetic plasma specimens with spiked-in proIAPP(1-67) synthetic peptide.

Results: Prior to ELISA development, the assay LOD was 33.7pM. Matrix effects were evaluated by calculating the percent-recovery from the spike and recovery analyses which ranged between 57% to 67%. Following optimization of the various assay experimental conditions, the LOD is now 3.2pM ± 1.3pM with percent-recovery values of 97.8% ± 14.2%. These values fall within the technically acceptable range of 80% to 120%. To determine whether the ELISA is capable of detecting proIAPP(1-67) synthesized by human islets, serial dilution of human islet lysates was performed. Plotting the concentration of proIAPP(1-67) versus total protein revealed a linear relationship. The ELISA had no detectable cross-reactivity with mature IAPP or proIAPP(1-48N) and detected <10% proIAPP(1-48C) immunoreactivity, all relative to proIAPP(1-67).

Conclusions: These data indicate a marked improvement in sensitivity for detection of proIAPP(1-67). Future directions include assessing the assay efficacy in T1D plasma specimens to determine whether the assay is functionally capable of measuring proIAPP(1-67) in this target population. Successful optimization for reliable proIAPP(1-67) measurement in T1D specimens will not only improve exploration of prohormone processing in diabetes but may also improve T1D prediction time and patient stratification in T1D clinical trials.

Megan Pawluk

GRADUATE STUDENT

GRANZYME B: A NOVEL THERAPEUTIC TARGET FOR RADIATION DERMATITIS

ABSTRACT #57

Supervisor: Dr. David Granville

Pawluk, Megan A[1,2], Hiroyasu, Sho[1,2], Nabai, Layla[1,2], Wadsworth, Brennan. J[2,3], Shen, Yue[1,2], Bennewith, Kevin L[2,3], Granville, David J[1,2,4]

Affiliations:
[1]International Collaboration On Repair Discoveries (ICORD), Vancouver Coastal Health Research Institute, UBC. [2]Department of Pathology and Laboratory Medicine, UBC. [3]BC Cancer Research Center. [4]British Columbia Professional Firefighters' Burn and Wound Healing Group.

Background/objectives: Radiation dermatitis (RD) appears in up to 95% of patients receiving radiation therapy for cancer treatment. Symptoms of RD include erythema, pruritus, pain, scaling, and ulceration with increased chance of infection. Severe symptoms can often delay or prevent additional courses of radiation treatments. Unfortunately, current treatments are not effective. Granzyme B (GzmB) was originally characterized as a pro-apoptotic serine protease secreted by cytotoxic lymphocytes to promote target cell apoptosis. It is now recognized that GzmB can be expressed and secreted by numerous immune as well as non-immune cell populations. Extracellular GzmB is dramatically elevated in a number of skin disorders, including atopic dermatitis, autoimmune blistering and scarring. Extracellular GzmB proteolytically cleaves numerous substrates that contributes to reduced epithelial barrier function, cleavage of basement membrane proteins, impaired matrix remodeling and scarring. GzmB is abundant in skin lesions of humans with RD. Based on these findings, I hypothesize that GzmB contributes to increased severity and delayed healing of RD through the cleavage of cell-cell junction proteins resulting in impairment of epidermal barrier function and increased inflammation.

Methods: GzmB expression was assessed in biopsies taken from patients exhibiting RD. The role of GzmB was investigated in an established murine model of RD, comparing GzmB knockout (GzmB-KO) to wild type (WT) mice. RD was induced in mice by applying a single 40 gray dose of radiation to the upper back. RD sections were blindly assessed and scored. Tissue samples were collected on days 4 (N=4) and 14 (N=14) and examined by histology and enzyme-linked immunosorbent assay (ELISA) for pro-inflammatory markers and GzmB levels.

Results: GzmB was markedly elevated in human skin tissue with RD when compared to healthy human skin tissue. GzmB-KO mice exhibited a significant decrease in RD severity compared to WT mice at day 4 ($p=0.03339$), day 6 ($p=0.01596$), day 8 ($p=0.007776$), day 10 ($p=0.01244$), and day 12 ($p=0.01408$) compared to WT mice. A significant reduction in skin redness (erythema), scaling, and crusted wounds 4-12 days post-radiation. Future studies will examine the utility of a topical GzmB inhibitor.

Conclusions: GzmB is abundant in human RD. GzmB may contribute to RD severity through E-cadherin cleavage and the subsequent loss of epithelial barrier function. GzmB may be a novel therapeutic target for the treatment of RD.

Tetiana Povshedna

GRADUATE STUDENT

RISK SCORES FOR AGE-RELATED COMORBIDITIES AND IMMUNE AGING MARKERS IN WOMEN OVER 45 YEARS OLD LIVING WITH OR WITHOUT HIV IN THE CARMA COHORT IN BRITISH COLUMBIA: A PRELIMINARY ANALYSIS

ABSTRACT #58

Background/objectives: The success of combination antiretroviral therapy for the treatment of HIV infection has increased the life expectancy of people living with HIV (PLWH). However, this population remains at higher risk of age-related comorbidities. There are limited data describing multiple comorbidity scores for risk prediction in women living with HIV (WLWH), who represent 23% of PLWH in Canada. Our objective was to compare risk scores and immune aging markers leukocyte telomere length (LTL) and mitochondrial DNA (mtDNA) content between WLWH and HIV-negative control women.

Methods: We retrospectively reviewed demographic and clinical data from 122 WLWH and 81 controls aged >45 years who had visits between 2008-2018 in the CARMA cohort. Framingham Risk Score (FRS), Atherosclerotic Cardiovascular Disease (ASCVD) Risk Score, aspartate aminotransferase-to-platelet ratio (APRI), and Canadian Association of Radiologists and Osteoporosis Canada (CAROC) score were calculated for both groups. Liver-related death (LRD) risk score, Veterans Aging Cohort Study (VACS) index, and Chronic Kidney Disease (CKD) prediction score are HIV-specific and were calculated for WLWH only. LTL and mtDNA content were measured by qPCR. Differences between groups were analyzed using Fisher's Exact or Mann-Whitney tests.

Results: WLWH were younger than controls (median[IQR] 53[49-57] vs. 55[50-59], p=0.028) but more likely to be current tobacco smokers (41% vs. 18%, p=0.008) and HCV+ (44% vs. 7%, p<0.0001). A larger proportion of WLWH had APRI>0.7 (21% vs. 4%, p<0.001), and moderate/high CAROC scores (39% vs. 19%, p=0.03) compared to controls, indicating higher risks for hepatic fibrosis and 10-year incident fracture, respectively. FRS and ASCVD, which both estimate 10-year risk of cardiovascular events, did not differ between groups. However, the two scores showed moderate agreement with respect to risk stratification (low/intermediate/high) for HIV-negative controls (n= 53, Cohen's kappa = 0.512), and only slight agreement for WLWH (n=74, Cohen's kappa = 0.147). Among WLWH, LRD risk score (n=47) was 5.5 [4.5-6.5], with 18.4% at medium-to-high risk of liver-related death, and CKD risk score was 7.0 [3.0-12.0] with 68% at high risk of disease. The VACS index predicts risk of 5-year mortality and was calculated for 119 WLWH. Among these, 32 (27%) had ≥20% risk while 46 (39%) has a <10% risk of 5-year mortality. Among WLWH who were current smokers (n=49), 22 (45%) had ≥20% risk of 5-year mortality. Finally, WLWH had shorter LTL than controls (6.6[6.1-7.3] vs. 7.4[6.8-8.0], p<0.0001), but we detected no difference in mtDNA content.

Conclusions: Compared to controls, WLWH had shorter LTL and higher risk scores for liver and bone, but not for cardiovascular diseases. The discrepancy between the two cardiovascular risk scores and the possible underestimation of risk highlight the importance of HIV-specific cardiovascular disease risk calculators. Comorbidity risk pattern for WLWH involves kidney, liver, and bone disease; smoking WLWH have a very high 5-year mortality risk. Further research is needed to better predict aging comorbidities and improve health outcomes in this population.

Supervisor: Dr. Helene Cote

Tetiana Povshedna[1], Maya Rosenkrantz[2], Anthony YY Hsieh[1], Jonathan Steif[3], Arianne Albert[4,5,6], Evelyn J Maan[5], Beheroze Sattha1,5, Ariel Nesbitt[2,5], Shanlea Gordon[4], Jerilynn Prior[2,4,7], Deborah M. Money[4,5,6], Melanie CM Murray[4,5,8], Neora Pick[4,5,8], Hélène C.F. Côté[1,4], for the CIHR Team Grant on Cellular Aging and HIV Comorbidities in Women and Children and the CARMA (Children and Women: AntiRetrovirals and Markers of Aging) cohort

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Medicine/Division of Endocrinology, UBC.
[3]Department of Mathematics, UBC.
[4]Women's Health Research Institute, British Columbia Women's Hospital.
[5]Oak Tree Clinic, British Columbia Women's Hospital.
[6]Department of Obstetrics and Gynecology, UBC.
[7]School of Population and Public Health, UBC.
[8]Department of Medicine, Division of Infectious Diseases, UBC.

Farnaz Sahragard

GRADUATE STUDENT

TESTING THE SAFETY OF AN OPTICAL SENSOR DESIGNED TO MONITOR SPINAL CORD HEMODYNAMICS

ABSTRACT #59

Supervisor: Drs. Babak Shadgan and Brian Kwon

Farnaz Sahragard[1,2], Amanda Cheung[1], Femke Streijger[1], Shahbaz Askari[1,3], Brian kwon[1,3,4], Babak Shadgan[1,2,3,4]

Affiliations:
[1]International Collaboration on Repair Discoveries (ICORD), UBC.
[2]Department of Pathology and Laboratory Medicine, UBC.
[3]School of Biomedical Engineering, UBC.
[4]Department of Orthopaedics, UBC.

Background/objectives: An implantable optical sensor, based on near-infrared spectroscopy (NIRS), was developed to monitor spinal cord hemodynamics and oxygenation after acute spinal cord injury (SCI). The ability to monitor these parameters within the injured cord will provide clinicians with critical information to optimize the hemodynamic management of the acutely injured patient. However, the effect of near-infrared light emission and contact compression of the NIRS sensor on the spinal cord tissue structure were not clear. This information is essential because even minimal heating or contact pressure from the NIRS sensor placed over the injured spinal cord may lead to further damage to the spinal cord tissue. We evaluated the safety of the custom-made NIRS sensor as it is essential prior to its clinical translation.

Methods: Six Yucatan miniature pigs received a T10 SCI. A miniaturized NIRS sensor prototype was place over the dura of the spinal cord and fixed with magnets and cross-connectors. After seven days of continuous data collection at 100 Hz, the NIRS sensor was removed, and the spinal cord tissue was examined. Histological assessment of the spinal cord revealed evidence of cellular damage at the NIRS sensor placement site in two animals. In-vitro experiments were performed to evaluate the possibility of heat damage caused by the NIRS sensor by using a digital thermometer with two probes, one probe was placed directly touching the light emitter of the NIRS sensor and the other probe was placed at a control site one centimeter away from the emitter. The temperature changes of the NIRS emitter after seven days of continuous operation at 100 Hz was measured and compared with the control site. This experiment was repeated three times. The NIRS sensor design, shape, and material were revised to minimize the sensor footprint, increase the sensor flexibility, and improve its clinical application. An in-vivo pilot experiment was performed on a Yucatan mini-pig with a T10 SCI to evaluate potential physical damage of the spinal cord tissue from placement of the refined NIRS sensor (version 5). The version 5 NIRS sensor was fixed on top of the spinal cord using only a fibrin sealant, Tisseel.

Results: *In-vitro* heat tests showed no heat generation by the NIRS sensor. The temperature measured from the emitter site of the NIRS sensor and control temperature probe was identical during the seven days. The in-vivo experiment revealed no signs of tissue damage, flattening, and indentation on the dorsal surface of the spinal cord where the version 5 NIRS sensor was fixed on the spinal cord with Tisseel.

Conclusions: Guided by histological analysis of the spinal cord tissue, we refined and examined the safety of a novel implantable spinal cord NIRS sensor for several hours. Applying this technology in human SCI patients requires further in-vivo studies to ensure the sensor is safe to use up to 14 days.

Chae Young Shin

GRADUATE STUDENT

INVESTIGATING THE TUMORIGENIC ROLE OF ARID1B LOSS IN ARID1A-MUTATED DEDIFFERENTIATED ENDOMETRIAL CARCINOMA

ABSTRACT #60

Background/objectives: In mammals, the role of the SWI/SNF chromatin remodeling complexes are to remodel chromatin by altering nucleosome position on the genome. As a result, SWI/SNF complexes are capable of epigenetically modifying cellular gene expression. In humans, the three main SWI/SNF complexes are BAF, PBAF, and GBAF. In particular, the BAF complex contains ARID1A or ARID1B, mutually exclusive DNA binding domains. Since the ARID1 components are critical for BAF function, they should be synthetically lethal, however they are often co-mutated across cancer types.

One such cancer type is dedifferentiated endometrial carcinoma (DDEC). Among endometrial carcinomas, DDEC is particularly aggressive and rare with a 5 year survival rate of only approximately 25%. DDEC consists of two components: the well differentiated low grade portion, and the portion that is thought to arise clonally from it, the high grade undifferentiated portion.

Due to some unique properties of DDEC, this cancer type is well suited for investigation of the tumorigenic properties of ARID1A/ARID1B dual loss. In some DDEC cases, the pattern arises that ARID1A and ARID1B are both missing in the undifferentiated portion, but only ARID1B is lost in the low grade component. This implies that in DDEC, ARID1B loss on top of ARID1A deficiency may be a driving factor in dedifferentiation, therefore tumorigenesis.

Supervisor: Dr. David Huntsman

Chae Young Shin[1,2], Raymond Zirui Feng[2], Valerie Lan Tao[2], Maxwell Douglas[2], Shelby Thornton[2], Lien Hoang[3], Yemin Wang[1,2], David Huntsman[1,2]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Molecular Oncology, British Columbia Cancer Research Centre.
[3]Anatomical Pathology at VGH.

Taras Shyp

GRADUATE STUDENT

ABSTRACT #61

Supervisor: Dr. Poul Sorensen

Taras Shyp[1,2], Rouhollah Mousavizadeh[1], Gian Luca Negri[3], Alberto Delaidelli[1,2], Andrii Vislovukh[1,2], Hai-Feng Zhang[2], Michael Lizardo[2], Poul Sorensen[1,2]

Affiliations:
[1]University of British Columbia.
[2]BC Cancer Research Institute.
[3]Genome Sciences Centre

SIX-TRANSMEMBRANE EPITHELIAL ANTIGEN OF THE PROSTATE 1 FACILITATES IRON TRANSPORT IN EWING SARCOMA TO SUPPORT MITOCHONDRIAL ACTIVITY

Background/objectives: Ewing sarcoma (EwS) is a highly aggressive bone-associated tumour in children and young adults with an early predisposition to metastasize and a dismal outcome for patients with advanced stages of the disease. EwS is characterized by pathognomonic oncogenic proteins, in 85-90% of cases EWSR1-FLI1. One of the previously described transcriptional targets of the fusion protein is six-transmembrane epithelial antigen of the prostate 1 (STEAP1), a member of the STEAP membrane-bound protein family that plays a crucial role in transient metal homeostasis. It was earlier described that STEAP1 potentiates invasiveness, tumour growth, and metastasis of EwS cells due to the oxidative induced stress phenotype. However, a precise functional role of STEAP1 in EwS remains elusive.

Methods: EwS cell lines with knockout (KO) or knockdown (KD) of STEAP1 were created to perform functional studies for elucidating the role of STEAP1 in EwS, including transient metal measurements, ROS measurements, mitochondrial activity evaluation. Additionally, formaldehyde cross-linking of STEAP1 protein complexes followed by immunoprecipitation (IP) and mass spectrometry (MS) analysis were performed to identify STEAP1 protein-protein interactions.

Results: We showed that STEAP1 functions as a metalloreductase in EwS, and STEAP1 KO cells have lower intracellular iron levels that positively correlate with the levels of ROS in STEAP1 cells, but not in STEAP1 KO cells. Moreover, a decreased level of mitochondrial ROS in STEAP1 KO/KD cells was accompanied by a reduced mitochondrial membrane potential that reflects the impaired mitochondrial activity. Furthermore, we discovered that STEAP1 KO cells have significantly lower levels of the mitochondrial labile iron pool. As a consequence, the functional activity of iron-sulfur containing proteins was impaired, namely, aconitase 2. Since, STEAP1 lacks a functionally important intracellular dehydrogenase domain, nonetheless, it possesses an intramembrane heme-containing group, we hypothesized that STEAP1 can form active complexes with other proteins that bear these electron-donating groups. Using IP with MS analysis, we identified another member of the STEAP family, STEAP2, as one of the top interactors for STEAP1. Moreover, using co-IP assay and proximity ligation assay we supported our findings.

Conclusions: This study is the first to demonstrate in vitro interaction between two members of the STEAP protein family. Furthermore, this newly discovered interaction uncovered the previously disputable functional role of STEAP1 in transition metal homeostasis and unveils alternative therapeutic strategies in EwS via using iron-chelating agents or compounds that promote a recently discovered iron-dependent form of cell death, ferroptosis. Moreover, STEAP2 in parallel to STEAP1 is a membrane-bound protein with high expression profile in malignant tumours, therefore can be viewed as a promising candidate for developing targeted therapies for EwS patients.

Tianna Sihota

GRADUATE STUDENT

CHARACTERIZATION OF A NOVEL TUMOR SUPPRESSOR AND RISK FACTOR IN LUNG ADENOCARCINOMA

ABSTRACT #62

Background/objectives: Lung cancer is the leading cause of cancer-related death worldwide, mainly due to the late diagnosis of disease. Identifying factors that increase one's susceptibility to lung cancer is therefore imperative for the development of early intervention strategies. While smoking is the major cause of lung cancer, genetics also play a critical role. However, the specific genes responsible for increasing lung cancer risk are still poorly understood. To identify mutated genes that confer a selective growth advantage, our group used whole exome sequencing to profile a panel of never-smoker patients with lung adenocarcinoma (LUAD), a type of non-small cell lung cancer. Among the most significantly mutated genes was SNF2 Histone Linker PHD RING Helicase (SHPRH), highlighting its candidacy as a potential tumor suppressor. SHPRH is an E3 ubiquitin ligase involved in DNA repair. Interestingly, SHPRH is located within a genetic locus that is associated with familial susceptibility to lung cancer. However, the functional characterization of SHPRH as a tumor suppressor gene and its role in LUAD has yet to be determined. The objective of this study is to evaluate the clinical and biological relevance of altered SHPRH expression on the development and progression of lung adenocarcinoma.

Methods: To determine the clinical relevance of SHPRH in LUAD, SHPRH copy number alterations and expression status were compared using the TCGA LUAD cohort (n=230). Patient survival plots were generated using the KM plotter database (n=672) to determine the association between SHPRH expression and disease outcome (median split, univariate cox regression). To functionally characterize the role of SHPRH in lung tumorigenesis, a doxycycline-inducible system was used to express wildtype SHPRH in LUAD cell lines with homozygous deletion (NCI-H1395) and mutation (NCI-H2009) of SHPRH. In preliminary sets of in vitro experiments (n=3), upon induction of SHPRH, clonogenic and soft agar colony formation assays were performed to assess for anchorage-dependent and -independent growth, respectively.

Results: Analysis of the TCGA LUAD cohort reveals that SHPRH is mutated or homozygously deleted in 7% of tumors and loss of SHPRH copy number is associated with having significantly less SHPRH expression ($p<0.0001$). Furthermore, LUAD patients with reduced SHPRH expression have significantly worse overall survival ($p=4.4e-7$). Initial results from the in vitro experiments suggest that expression of wildtype SHRPH in homozygous deletion and mutant SHPRH cell lines may reduce their colony growth.)

Conclusions: This initial data suggests that inactivation of SHPRH may negatively affect patient outcomes and may function to enhance the tumorigenic potential of LUAD. However, continued investigation is required – in vitro using tumorigenicity assays and in vivo using mouse xenografts – to determine the biological relevance of SHPRH in LUAD pathogenesis. Understanding the role of SHPRH may lead to it becoming an important clinical biomarker for identifying individuals with an increased risk of developing LUAD and to help improve disease outcomes.

Supervisor: Dr. William Lockwood

Tianna S Sihota[1,2], Amy Nagelberg[1,2], Rocky Shi[2,3], Fraser D Johnson[2,3], and William W Lockwood[1,2,3]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC.
[2]Department of Integrative Oncology, BCCRC.
[3]Department of Interdisciplinary Oncology, UBC

Marie-Soleil Smith

GRADUATE STUDENT

ROBUST TOBACCO SMOKING SELF-REPORT IN TWO PAN-CANADIAN COHORTS: PREGNANT WOMEN OR MEN AND WOMEN LIVING WITH OR WITHOUT HIV

ABSTRACT #63

Supervisor: Dr. Hélène Côté

Marie-Soleil R. Smith[1,2], Sara Saberi[1,2], Abhinav Ajaykumar[1,2], Mayanne MT Zhu[1], Izabelle Gadawski[1,2], Beheroze Sattha[1], Evelyn J Maan[3,4], Julie Van Schalkwyk[3,4,5], Chelsea Elwood[3,5], Neora Pick[3,5,6], Melanie C.M. Murray[3,5,6], Isabelle Boucoiran[7], Deborah M. Money[3,4,5], Hélène C.F. Côté[1,2,3], and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA)

Affiliations:
[1]Department of Pathology and Laboratory Medicine, UBC. [2]Centre for Blood Research, UBC. [3]Women's Health Research Institute. [4]Oak Tree Clinic, Vancouver. [5]Department of Obstetrics and Gynaecology, UBC. [6]Department of Medicine, Division of Infectious Diseases, UBC. [7]Department of Obstetrics and Gynaecology, CHU Sainte-Justine, Université de Montréal, Montreal

Background/objectives: Stigma associated with tobacco smoking, especially during pregnancy, may lead to underreporting and possible bias in studies relying on self-reported smoking data. The objective of this study was to evaluate concordance between self-reported smoking and concentrations of plasma cotinine, a biomarker of smoking, among participants enrolled in two related pan-Canadian cohorts: pregnant women living with HIV (LWH) and control pregnant women enrolled in the Children and Women: Antiretrovirals and Markers of Aging (CARMA)-PREG cohort, as well as women and men LWH or not, enrolled in the CARMA-CORE cohort.

Methods: A retrospective controlled sub-study on a subset of participants enrolled in the two CARMA cohort studies was conducted. Plasma cotinine was measured by ELISA; for pregnant women, we used specimens collected in the third trimester. The concordance between smoking status determined by plasma cotinine levels >5ng/mL and self-report was examined. As this study involved secondary use of data, participants and research staff were not aware that cotinine levels would be measured.

Results: A total of 100 pregnant women (76 LWH and 24 negative controls) in their third trimester, and 100 men and non-pregnant women (43 LWH and 57 negative controls) were included. Among all participants, 93 (47%) were self-reported smokers: 43 pregnant women (49% LWH and 25% negative controls), and 50 men and non-pregnant women (58% LWH and 44% negative controls). The odds of discordance between self-reported smoking and cotinine levels were not significantly different between self-reported smokers and non-smokers, nor between pregnant women and others, but were significantly different, regardless of self-reported status, between people LWH and negative controls. The overall concordance between plasma cotinine and self-reported data among all participants was 94% with a sensitivity and specificity of 90% and 96%, respectively.

Conclusions: Taken together, these data demonstrate that participant surveying in a non-judgemental context can lead to accurate and robust self-report smoking data among both persons LWH and not, including in the context of pregnancy. Our results suggest that future studies should ensure a safe and non-judgemental setting for study participants, to obtain reliable self-report data.

Darcy Sutherland

GRADUATE STUDENT

SYNTHETIC ANTIMICROBIAL PEPTIDES KILL MULTI-DRUG RESISTANT PATHOGENS IN VITRO

ABSTRACT #64

Background/objectives: The discovery of antibiotics revolutionized medicine and lead to a significant increase in global life expectancy. However, it was soon realized that bacteria could develop resistance strategies to antibiotics. Because of this rapidly escalating global health concern, research into antimicrobial peptides (AMPs) has been gaining in popularity. AMPs are small molecules of the innate immune system and may be found within all organisms. In comparison to conventional antibiotics, which have specific gene-encoded or structural targets, AMPs may play multiple roles against infection, acting directly on the bacterial cell membrane or through modulation of the host immune system. Our lab has developed a cross-disciplinary approach to rapidly discover and validate novel AMPs for activity against antibiotic resistant pathogens. We hope to ultimately develop AMPs into novel therapeutics to address the emergence of multi-drug resistant (MDR) bacteria, also known as “superbugs”.

Methods: We have assembled a computationally driven platform to discover AMPs from publicly available genomics resources. We have structured our analysis pipeline to identify AMP sequences using gene homology-based methods and subsequent prioritization of AMPs using a deep-learning machine-learning classifier, ‘AMPlify’. We additionally mutated select AMPs to increase their net charge in an attempt to increase their antimicrobial potency. Top-scoring AMPs were selected for synthesis and validation *in vitro*.

(Antimicrobial susceptibility testing): AMPs were serially diluted from 256 to 0.5 ug/mL in a 96-well format before being combined with a standardized inoculum. Minimum inhibitory concentration (MIC) values were reported at the concentration in which provided no visible growth following overnight incubation, as recommended by the Clinical Laboratory Standards Institute. Adjacent wells were plated onto non-selective media to determine the minimum bactericidal concentration (MBC).

Results: We present nine novel AMPs with demonstrated antimicrobial activity against a variety of MDR bacteria, four of which provided MIC/MBC values of 4-16 ug/mL against three strains of MDR E. coli.Importantly, all presented mutant AMPs demonstrated an increase in antibacterial activity. In each instance, increasing the AMP’s positive charge was sufficient to observe an increased shift in antibacterial activity. This is evident when considering the most positively charged AMPs (>= +4), as these were found to be the most potent of our experimental set.

Conclusions: This represents a strong validation of our computationally driven AMP discovery pipeline to produce novel AMPs. Encouragingly, many of our AMPs demonstrated similar antibacterial activity against the MDR isolates as the “antibiotic susceptible” ATCC control strains. This implies that the target of our AMPs is a mechanism not previously selected for with conventional antibiotics; and thus, provide a viable solution to the increasing emergence of MDR pathogens.

Supervisor: Drs. Inanc Birol & Mel Krajden

Darcy Sutherland[1,2,3], S Austin Hammond[1], Chenkai Li[1,4], René L Warren[1], Caren Helbing[5], Titus Wong[5], Linda Hoang[2,3], Mel Krajden[2,3] and İnanç Birol[1,2,3,7]

Affiliations:
[1]Canada’s Michael Smith Genome Sciences Centre, BCCA. [2]Public Health Laboratory, BCCDC. [3]Department of Pathology and Laboratory Medicine, UBC. [4]Bioinformatics Graduate Program, UBC. [5]Department of Biochemistry and Microbiology, University of Victoria. [6]Medical Microbiology Laboratory, VGH. [7]Department of Medical Genetics, UBC

Busra Turgu

GRADUATE STUDENT

ABSTRACT #65

Supervisor: Dr. Poul Sorensen

Busra Turgu[1,2], Fan Zhang[3], Amal El-Naggar[1,4,5], Gian Luca Negri[6], Melanie Kogler[7], Luigi Tortola[7,8], Fraser Johnson[1], Tony Ng[4], Amy Li[1], Donald Yapp[9], William Lockwood[4,10], Daniel Martinez[11], John M Maris[12], Mads Daugaard[3], Josef M Penninger[7,13], Christopher S Hughes[1], Poul H Sorensen[14,15]

Affiliations:
[1]Dept of Molecular Oncology, BCCRC. [2]Interdisciplinary Oncology Graduate Program, Faculty of Medicine, UBC. [3]Dept of Urological Sciences, The Vancouver Prostate Centre, UBC. [4]Dept of Pathology and Laboratory Medicine, UBC. [5]Department of Pathology, Faculty of Medicine, Menoufia University, Shibin El Kom, Egypt. [6]Canada's Michael Smith Genome Sciences Centre, BCCA. [7]Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austria. [8]Dept of Biology, Institute of Molecular Health Sciences, ETH Zurich, Switzerland. [9]Dept of Experimental Therapeutics, BCCRC. [10]Department of Integrative Oncology, BCCRC. [11]Dept of Pathology, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [12]Division of Oncology and Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, USA. [13]Dept of Medical Genetics, Life Science Institute, UBC. [14]Dept of Molecular Oncology, BCCRC. [15]Dept of Pathology and Laboratory Medicine, UBC.

HACE1 BLOCKS HIF1A ACCUMULATION UNDER HYPOXIA IN A RAC1 DEPENDENT MANNER

Background/objectives: Uncovering the mechanisms that underpin how tumor cells adapt to microenvironmental stress is essential to better understand cancer progression. The HACE1 gene is a tumor suppressor that inhibits the growth, and metastasis of cancer cells. However, the direct regulatory pathways whereby HACE1 confers this tumor-suppressive effect remain to be fully elucidated. The most well-characterized E3 ligase target of HACE1 is RAC1, which was originally identified as an oncogene. HIF1alpha is a transcription factor that is a key regulator of metastasis under hypoxia. HIF1α induction under hypoxia requires activation of RAC1. Despite these established relationships between HACE1 and RAC1, and RAC1 and HIF1α, potential links between HACE1 and HIF1α have not been investigated.

Methods: To probe the pathophysiological significance, we investigated the relative levels of HACE1 and HIF1α protein in WT cases (n=3) compared with patient-matched normal kidney adjacent to tumor tissues. To further assess the link between HACE1 and HIF1α in human tumors, tissue microarrays (TMAs) consisting of WT (9 cases) and different childhood sarcomas (18 cases, including 5 Ewing sarcoma cases, 3 alveolar rhabdomyosarcoma cases, 4 embryonal rhabdomyosarcoma cases, and 6 synovial sarcoma cases) were subjected to IHC for HACE1 and HIF1α expression. In addition to in vitro expreiments, to investigate the link in vivo, we used a previously described mouse lung cancer model of Hace1 inactivation in which deletion of Rac1 and expression of oncogenic KRasG12D can be simultaneously induced by Adeno-Cre administration. IHC was performed to assess HIF1α expression in FFPE lung tumor tissues obtained from 8 and 16-week-old KRasG12DHace1+/+Rac1+/+, KRasG12DHace1–/–Rac1+/+, KRasG12DHace1+/+Rac1fl/fl, and KRasG12DHace1–/–Rac1fl/fl mice.

Results: In this report, we establish a link between HACE1 and the major stress factor, hypoxia-inducible factor 1 alpha (HIF1α). We find that HACE1 blocks the accumulation of HIF1α during cellular hypoxia through decreased protein stability. This property is dependent on HACE1 E3 ligase activity and loss of Ras-related C3 botulinum toxin substrate 1 (RAC1), an established target of HACE1 mediated ubiquitinylation and degradation. In vivo, genetic deletion of Rac1 reversed the increased HIF1α expression observed in Hace1-/- mice in murine KRasG12D-driven lung tumors. An inverse relationship was observed between HACE1 and HIF1α levels in tumors compared to patient-matched normal kidney tissues, highlighting the potential pathophysiological significance of our findings.

Conclusions: Together, our data uncover a previously unrecognized function for the HACE1 tumor suppressor in blocking HIF1α accumulation under hypoxia in a RAC1-dependent manner.

Henry West

GRADUATE STUDENT

THE ROLE OF TISSUE FACTOR IN VIRAL INFECTION AND PATHOLOGY

ABSTRACT #66

Supervisor: Dr. Ed Pryzdial

Henry West[1,3], Alex Leatherdale[1,3], Bryan Lin[1,3], Michael Sutherland[1,3,4], Ed Conway[1,2,3], Ed Pryzdial[1,3,4]

Affiliations:
[1]Centre for Blood Research, Life Sciences Institute, University of British Columbia.
[2]Division of Hematology, Department of Medicine, University of British Columbia.
[3]Department of Pathology and Laboratory Medicine, University of British Columbia.
4Canadian Blood Services, Centre for Innovation, University of British Columbia

Background/objectives: Wide-ranging viral pathologies involve interconnected hemostatic imbalances and inflammation, such as AIDS, hemorrhagic fever and COVID-19, caused by human immunodeficiency virus (HIV), dengue virus (DENV) and SARS-CoV-2 infection, respectively. Here, we examine the interplay between coagulation and inflammation focusing on tissue factor (TF), a transmembrane protein cofactor that initiates coagulation and critical cell signaling, and a bridge between coagulation and inflammation. Our studies have discovered TF on the envelope (the outer membrane) of several viruses. Using oral herpes (HSV1) as a model enveloped virus, viral TF strikingly enhanced infectivity in vitro and in vivo through cell signaling and effectively initiated clotting. Since TF is broadly expressed on many cell types, we hypothesize that TF is ubiquitous on the surface of enveloped viruses and plays a role in viral pathology and infection.
Goal 1: Demonstrate TF antigen and activity on HIV and DENV, other important enveloped viruses.
Goal 2: Evaluate COVID-19 patient serum for TF-induction on endothelial cells.

Methods: **Goal 1:** To identify TF antigen on the virus surface, immunogold electron microscopy (IEM) was used. TF-dependent activation of coagulation factor (F) X to FXa by FVIIa in the presence of calcium was followed chromogenically by the addition of lab strain purified (lsp)-virus as the only source of TF. Recalcified plasma clotting assays were also conducted with lsp-virus as the only source of TF.
Goal 2: A cell based fluorogenic assay was developed to assess the effect of COVID-19 patient sera on the activation and thrombin generation potential of an endothelial cell monolayer (EA.hy926). Cells were treated with COVID-19 patient or healthy control sera for 1 hour and then washed. Normal human plasma was layered on and the reaction initiated with calcium. Thrombin generation was followed through cleavage of a thrombin-specific fluorogenic substrate.

Results: **Goal 1**: TF on the surface of lsp-DENV and patient plasma derived-HIV was identified by immunogold electron microscopy. Experiments are ongoing with the corollary ppd-DENV and lsp-HIV. Initiation of plasma clotting was facilitated by lsp-HIV and lsp-DENV. Utilizing purified proteins, lsp-HIV and lsp-DENV also facilitated FVIIa-dependent FXa generation. Both assays were inhibited by TF-specific inhibitors.
Goal 2: COVID-19 patient serum induced more thrombin generation by endothelial cells compared to treatment with healthy control serum. The enhanced activity was paralleled by increased cell surface TF antigen. A specific TF antibody inhibited thrombin generation.

Conclusions: Our data support the ubiquity of functional TF on the surface of viruses, and a central role for TF in the pathobiology of virus infection. Further studies are underway to investigate the role of TF in the HIV infection cycle to explain the early onset thrombosis people with AIDS are experiencing. Additionally, we are dissecting the proinflammatory signaling mechanisms to elucidate COVID-19 sera-mediated induction of TF on endothelial cells.

Nancy Yang

GRADUATE STUDENT

THE IMPACT OF CHRONIC AND LATENT VIRAL INFECTIONS ON AGING IN PEOPLE LIVING WITH HIV

ABSTRACT #67

Supervisor: Dr. Hélène Côté

Nancy Yang[1,2], Zhuo Chen[1], Hélène CF Côte[1,2], for the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA cohort)

Affiliations:
[1]University of British Columbia (UBC), Vancouver, BC
[2]Centre for Blood Research, UBC, Vancouver, BC

Background/objectives: Over 38 million people are currently living with HIV. Despite effective antiretroviral therapies that have prevented transmission and increased lifespan, these individuals experience faster biological aging and immune senescence relative to those living without HIV. HIV, cytomegalovirus (CMV), hepatitis C virus (HCV), Epstein-Barr virus (EBV), and human herpesvirus 8 (HHV-8) have been associated with shortened telomeres in immune cells. These viruses cause lifelong infections, promoting immune weakening in people living with HIV. Despite knowing how these viruses individually contributing to immunological aging, few studies have looked at more than two infections at a time -revealing a gap in knowledge about the effect of having multiple viruses on aging. Our objectives are to characterize the number and type of chronic/latent viral infections in a cohort of people living with and without HIV, and determine their association with markers of immune aging.

Methods: 170 participants have been semi-randomly selected to attempt to balance for sex, HIV status, and age. Whole blood has been processed into plasma, serum, and live peripheral blood mononuclear cells. Serology for HSV-1 (herpes simplex virus 1) and HSV-2 (herpes simplex virus 2) are done at the BC Centre for Disease Control. Serological assays for CMV, EBV, and HHV-8 IgG are done using commercial ELISA kits. HCV status will be determined by self-report. Average leukocyte telomere length (LTL) is quantified via monochrome multiplex qPCR. Univariate comparisons between sex, age, HIV status, LTL, and number of viruses, will be assessed with unpaired t-tests or Mann-Whitney tests. A multivariable linear regression model of LTL with Tukey's correction will be done.

Results: EBV appears to have a high prevalence in both HIV- and HIV+ groups. The likelihood of infection of a particular virus seems to increase with age. HSV-1 infection appears to travel with EBV infection in the HIV- group, and HSV-2 appears to travel with EBV in the HIV+ group. All HIV+ participants have at least one other viral infection, and all participants older than 30 have more than 1 other infection (excluding HIV). It appears that HIV+ participants may have more viruses compared to the HIV- group.

Conclusions: The preliminary data appears to depict that it is likely that HIV+ participants have at least one other virus, that older participants may be infected with more viruses, and that some viral infections may increase with HIV co-infection. Further experimentation is needed to balance the data set with respect to age, sex, and HIV status. Better understanding the accelerating aging experienced by people living with HIV is crucial to improving quality and life and health care burden.

Joyce Zhang

GRADUATE STUDENT

LOW-GRADE SEROUS OVARIAN CANCERS MODELLED WITH HUMAN FALLOPIAN TUBE ORGANOIDS AND SINGLE CELL SEQUENCING

ABSTRACT #68

Background/objectives: Ovarian cancers are the most common gynecologic malignancies. Low grade serous ovarian carcinoma (LGSOC) is a rare tumour, accounting for 5% of all ovarian cancer cases. Most of LGSOCs are characterized by high fatality rates over the long term, with only 10-20% of women surviving 10 years after diagnosis, due suboptimal response to current chemotherapies. Understanding the molecular events is crucial for developing more informed therapeutic options. LGSOC harbours a relatively stable genome, with common activating mutations in BRAF, KRAS and NRAS. Recently, NRAS mutation (Q61R) were found to co-exist with EIF1AXmutations (G8E) in LGOSC, and the two mutated proteins functionally cooperate. Low incidence of this disease means it is poorly understood, and the resulting lack of available models further limits the study of the underlying mechanisms. We therefore propose to utilize organoid cultures, which consist of 3D multicellular units that resemble in vitro a tissue or organ of body. We aim to elucidate molecular events underpinning LGSOC, specifically how NRAS(Q61R) and eIF1a (G8E) mutations co-operate to drive early stages of tumorigenesis, with organoid system and single-cell RNA sequencing (scRNA-seq) technologies.

Methods: To reflect genetic background and cell of origin of LGSOC, NRAS(Q61R) and eIF1a(G8E) mutant proteins were overexpressed via lentiviral transduction in primary normal human Fallopian tube tissues. After allowing organoids to establish, gene expression alterations were resolved with scRNA-seq 2 weeks after transduction. Organoid cytology was assessed for signs of transformation. Patient-derived tumor organoids (PDTOs) were cultured to assess how well our LGSOC-modelling organoids (LMOs) recapitulate the histological features of patient tumours.

Results: LMOs showed cytologic signs of transformation (increased nuclear/cytoplasmic ratio, prominent nucleoli, cellular pleomorphism). Papillary structures, a major histologic characteristic of LGSOC were observed in LMOs. PDTOs showed similar cytological features and organization as LMOs. From scRNA-seq, we identified genes up-regulated in double-mutant compared to single-mutant organoids such as CA125 and TACSTD2. CA125 is one of the earliest identified biomarkers for ovarian cancer and has remained to be a useful serum marker despite limited sensitivity and specificity; whereas TACSTD2 overexpression has been found to correlate with a chemo-resistant, aggressive malignant phenotype.

Conclusions: We established a novel model that largely recapitulated LGSOC cytology by introducing co-occurring mutations into Fallopian tube tissues. Genes upregulated in double mutants included well-characterized biomarker (CA125) and a potential biomarker or therapeutic target (TACSTD2). Our work will be crucial for developing more targeted treatment options.

Supervisor: Dr. David Huntsman

Joyce Zhang[1,2], Dawn Cochrane[1], Kieran Campbell[1], Minh Bui[1], Germain Ho[1], Genny Trigo[1], Winnie Yang[1], Cindy Shen[1], Sorab Shah[1], David Huntsman[1,2]

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

Guangze Zhao

GRADUATE STUDENT

KNOCKDOWN OF NUCLEAR FACTOR OF ACTIVATED T-CELLS 5 PROMOTES THE COXSACKIEVIRUS B3-INDUCED MYOCARDITIS

ABSTRACT #69

Supervisor: Dr. Decheng Yang

Guangze Zhao[1,2], Mary Huifang Zhang[1,2], Fione Yip[1,2], Sana Aghakeshmiri[1,2], Terry Chen[1,2], Christoph Kuper[3], Decheng Yang[*1,2]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada
[2] Centre for Heart Lung Innovation, St. Paul's Hospital, Vancouver, Canada
[3] Department of Physiology, University of Munich, Munich, Germany

Background/objectives: Viral myocarditis is an inflammatory heart disease caused by viral infection and CVB3 (coxsackievirus B3) is the predominant pathogen for myocarditis. Upon infection, CVB3 modulates various cellular signalling pathways, leading to cell cycle arrest and programmed cell death. ICDs (Intercalated Discs) are substantial connections maintaining cardiac structure and mediating signal communication among cardiomyocytes. NFAT5 (Nuclear factor of activated T-cells 5) is a transcription factor that was shown to regulate some ICD proteins, and was cleaved by CVB3 viral proteases during infection. In order to delineate the interplay between NFAT5 and ICD structure in CVB3 infected conditions, we generated a conditional cardiac-specific NFAT5 KO (knockout) mouse model and verified that NFAT5 KO mice are more susceptible to CVB3 infection. Thus, we hypothesize that CVB3-induced cleavage of NFAT5 leads to the destruction of ICD proteins, which results in damages of cardiomyocytes and contributes to viral pathogenesis.

Methods: In the study, we generated a specific mouse model by using a tamoxifen-inducible Cre-loxp system to induce NFAT5 KO in the heart. The littermate Cre negative NFAT5flx/flx mice were used as controls. Two weeks after tamoxifen induction and then CVB3 infection, mouse heart as well as other organs were harvested to confirm the KO of NFAT5 by semi-qPCR and western blot. The replication of CVB3 in the heart was detected by qPCR and western blot to detect viral RNA and VP1 protein, respectively. Meanwhile, H&E staining was used to determine the inflammatory response in different mice organs.

Results: The results showed that the protein level of NFAT5 in the heart dropped significantly compared to that in other organs. Moreover, the transcription levels of desmoplakin, connexion 43 and N-cadherin genes in the ICD structure were differentially regulated by NFAT5. Also, more cardiac damages and inflammatory infiltrates were observed in CVB3-infected NFAT5 KO mice than in control mice, and this difference can only be observed in the heart tissue, but not in other organs like pancreas.

Conclusions: Taken together, our study suggests that the cardiac-specific NFAT5 KO mice are more susceptible to CVB3 infection and have more severe myocarditis compared to the non-KO group.

Tom Cheng

POSTDOCTORAL FELLOW

CHIMERA TRAUMATIC BRAIN INJURY IN A MOUSE MODEL OF TAUOPATHY

ABSTRACT #70

Background/objectives: Traumatic brain injury (TBI) is a major cause of worldwide death and disability. Moderate/severe TBI may increase risk of neurodegeneration in long-term, including Alzheimer's disease. To study the relationship between TBI and neurodegeneration, we have previously designed an animal model of impact-acceleration TBI called Closed Head Injury Model of Engineered Rotational Acceleration (CHIMERA). In this study, we induced moderate/severe TBI (msTBI) to rTg4510 mice, a mouse model of tauopathy. We aim to study if CHIMERA TBI exacerbates tau pathologies.

Methods: A single msTBI was induced at 4.0 J impact energy to male rTg4510 mice, at 4 months of age. The sham injury group received all procedures except for impact. Brain tissues were harvested 2 months post-injury and analysed using immunohistochemistry and Western blotting for multiple tau epitopes, neuroinflammation, and axonal injury. Blood samples were collected biweekly for biomarker analyses.

Results: Compared to sham controls, TBI animals showed a significantly increased duration of loss of righting reflex. Histological analyses indicated significant microgliosis (Iba1) and axonal injury (Neurosilver) at white matter, but no significant change in neuronal number (NeuN), astrocytes (GFAP), endothelial cells (CD31), or blood brain barrier integrity (IgG). TBI animals had increased p-tau immunoreactivity for PHF1 but not other tau epitopes (CP13, AT8, MC1, or RZ3). TBI animals had a reduced ratio of p-GSK-3beta (S9) to GSK-3beta, suggesting TBI animals had less of the inactive form of the tau-degrading enzyme GSK-3beta. TBI animals had significantly higher levels of Capthesin D and P62 levels suggesting impaired autophagosome/lysosome pathways.

Conclusions: Our preliminary findings suggest that TBI may lead to chronic tauopathy by altering GSK-3beta activity and impairing autophagy/lysosomal functions. Validation studies are underway.

Supervisor: Dr. Cheryl Wellington

Wai Hang Cheng[1], Grace Hu[1], Jianjia Fan[1], Honor Cheung[1], Jennifer Cooper[1], Carlos J Barron[1], Peter A Cripton[2], David Vocadlo[3], Cheryl L Wellington[1]

Affiliations:
[1]Department of Pathology and Laboratory Medicine, The University of British Columbia.
[2]Departments of Mechanical Engineering and Orthopaedics, The University of British Columbia. International Collaboration on Repair Discoveries, The University of British Columbia.
[3]Department of Chemistry, Simon Fraser University

Mona Khorshidfar

POSTDOCTORAL FELLOW

QUALITY MEASUREMENTS OF APHERESIS PLATELET CONCENTRATES FROM DONORS WHOSE PLATELETS REPEATEDLY SHOW LOW PH OVER STORAGE

ABSTRACT #71

Supervisor: Dr. Dana Devine

Mona Khorshidfar[1], Katherine Serrano[1,2], Mary Huang[3], Dana V. Devine[1,2]

Affiliations:
[1]Center for Blood Research, University of British Columbia
[2]Canadian Blood Services Center for Innovation
[3]Canadian Blood Services

Background/objectives: Platelet concentrate storage at room temperature is limited to 7 days due to a gradual loss of quality and the potential for bacterial growth. The pH of stored platelets is monitored at expiry as a surrogate measure of platelet quality during storage. Much research has been done on platelet preparation and storage; however, donor factors that contribute to platelet quality should also be considered. We investigated and compared characteristics of apheresis platelet concentrates (APC) from donors whose outdate pH is repeatedly low (pH≤6.7), and normal controls (pH>6.7).

Methods: APC quality measurements were made at multiple storage time-points, including pH and lactate by blood gas analyzer. CD62P expression, annexin V binding, platelet microvesicles (PMV) counts, and platelet response to ADP were analysed by flow cytometry. Platelet morphology was assessed with fixed platelets viewed using phase contrast microscopy. Bacterial testing (BacT/ALERT) was done on the last day of sampling.

Results: From quality control data we identified 10 donors whose platelet pH was always low and 39 donors whose platelet pH was low on some donations but not others among 12,066 donors. Compared to control (storage day 7), always low pH APC, showed significant increases in lactate (25.5 ± 6.3 mmol/L vs. 13.6 ± 1.7 mmol/L), CD62P expression (66.3 ± 0.2% vs. 41.4 ± 0.1%), annexin V binding (4.45 ± 0.05% vs. 2.26 ± 0.01%) and PMV count (3297 ± 1861 vs. 1717 ± 1698) while ADP response was decreased (19.5 ± 0.1% vs. 31.6 ± 0.1%) ($p<0.05$). Microscopy confirmed many balloon forms, activated and aggregated platelets in always low pH APC compared to controls. No contamination was detected.

Conclusions: Glycolysis rates and platelet activation levels are higher for low pH donors suggesting these platelets may be of lower quality. There appear to be donor factors which affect the bioenergetics of platelets during storage. Consideration should be given to alternative strategies for managing donations from these donors particularly if they have a rare HLA type.

Hai-Feng Zhang

POSTDOCTORAL FELLOW

PROTEOMIC SCREENS FOR SUPPRESSORS OF ANOIKIS IDENTIFY IL1RAP AS A NEW SURFACE TARGET FOR IMMUNOTHERAPY IN EWING SARCOMA

ABSTRACT #72

Background/objectives: Cancer cells must overcome anoikis (detachment-induced death) to successfully metastasize. Using proteomic screens, we found that distinct oncoproteins upregulate IL-1 receptor accessory protein (IL1RAP) to suppress anoikis. IL1RAP is directly induced by oncogenic fusions of Ewing sarcoma (EwS), a highly metastatic childhood sarcoma. IL1RAP inactivation triggers anoikis and impedes metastatic dissemination of EwS cells. Mechanistically, IL1RAP binds the cell surface system Xc- transporter to enhance exogenous cystine uptake, thereby replenishing cysteine and the glutathione antioxidant. Under cystine depletion, IL1RAP induces cystathionine gamma lyase (CTH) to activate the transsulfuration pathway for de novo cysteine synthesis. Therefore IL1RAP maintains cyst(e)ine and glutathione pools which are vital for redox homeostasis and anoikis resistance. IL1RAP is minimally expressed in pediatric and adult normal tissues, and human anti-IL1RAP antibodies induce potent antibody-dependent cellular cytotoxicity of EwS cells. Therefore, we define IL1RAP as a new cell surface target in EwS, which is potentially exploitable for immunotherapy.

Supervisor: Dr. Poul Sorensen

Hai-Feng Zhang[1,2], Christopher S Hughes[1,2], Wei Li[3], Jianzhong He[4], Didier Surdez[5], Amal M El-Naggar[1,2], Hongwei Cheng[2], Anna Prudova[2,6], Alberto Delaidelli[1,2], Gian Luca Negri[6], Xiaojun Li[2], Maj S Ørum-Madsen[7], Michael M Lizardo[2], Htoo Zarni Oo[7], Shane Colborne[6], Taras Shyp[1,2], Renata Scopim Ribeiro[1,2], Colin A Hammond[8], Anne-Chloe Dhez[1,2], Sofya Langman[1,2], Jonathan KM Lim[1,2], Sonia HY Kung[7], Amy Li[1,2], Anne Steino[2], Mads Daugaard[7], Rimas J Orentas[9], Li-Yan Xu[4], Gregg B Morin[6,10], Olivier Delattre[5], Dimiter S Dimitrov[3], Poul H Sorensen[1,2]

Affiliations:
[1]Department of Pathology and Laboratory Medicine,UBC. [2]Department of Molecular Oncology, BC Cancer Agency. [3]Center for Antibody Therapeutics, Division of Infectious Diseases, Department of Medicine, University of Pittsburgh Medical School, 3550 Terrace St., Pittsburgh, USA. [4]Institute of Oncologic Pathology, Shantou University Medical College, Shantou, Guangdong China. [5]Centre de recherche de l'Institut Curie, Paris 75248, France. [6]Canada's Michael Smith Genome Sciences Centre. [7]Vancouver Prostate Centre. [8]Terry Fox Laboratory, BCCA. [9]Seattle Children's Research Institute, Seattle, WA 98101, United States. [10]Department of Medical Genetics, UBC

Narges Hadjesfandiari

RESEARCH ASSOCIATE

ABSTRACT #73

Supervisor: Dr. Dana Devine

Narges Hadjesfandiari[1,2], Katherine Serrano[1,2], Elena Levin[1,2], Caesar DiFranco[2] and Dana Devine[1,2]

Affiliations:
[1]Centre for Blood Research, University of British Columbia,
[2]Centre for Innovation, Canadian Blood Services

FATTY WHOLE BLOOD DONATIONS AND HIGH RED BLOOD CELL STORAGE HEMOLYSIS

Background/objectives: Different donor factors such as sex, age, ethnicity, and frequency of donation have been previously associated with hemolysis level in red cell concentrates (RCCs). This study explored a large national blood services database to investigate any significance of the donor's body mass index (BMI) in storage hemolysis. Lipemia has been previously investigated in relation to RBC remodelling and intracellular reactive oxygen species. In the second part of this study, we measured triglyceride levels in the donors whose repeat donations in our database exceeded the acceptable level of hemolysis at outdate (referred to as repeat high hemolyzers) and compared them with the levels in control normal donors.

Methods: We investigated the quality control data set of 1,967 RCCs donated by 17 to 23-year-old donors from Jan 2015 to December 2019 at a national blood service. This is the age range that donors' weight and height are recorded at the time of donation in our jurisdiction. Percent hemolysis measured during routine quality control testing at the expiry of RCC was linked with the donors' BMI in male and female donors, separately. For the second part, in a data set of 19,700 RCC donations, 13 male repeat high hemolyzers were identified. These donors had at least two failed donations (hemolysis level ≥0.8% at outdate) with no history of donations with normal hemolysis levels in our data set. Plasma triglyceride levels in these donors and 11 male control donors were measured. Analyses of significant differences between means/medians of groups of interest were performed using parametric and nonparametric statistics.

Results: For males (n=932), average % hemolysis at expiry was 0.24±0.1% in the donors with BMI <25 (underweight, normal or heavy weight) versus 0.28±0.2% in donors with BMI >25 (overweight and obese), $p<0.001$. Percent failed donations (hemolysis level ≥0.8%) was 0.5% in the males with BMI <25 versus 2% in males with BMI >25. On the other hand, there was no significant difference between the % hemolysis level of the females with BMI <25 and those with BMI >25; average % hemolysis was 0.24 ± 0.1% in both female groups (n=1035).

Plasma samples from repeat high hemolyzers consistently had a turbid appearance. Repeat high hemolyzers had a significantly higher triglyceride level (3.6±1.5 mmol/L) than the control donors (1.6±0.93 mmol/L), $p<0.01$. Triglyceride levels in female repeat high hemolyzers and controls were not different; however, the sample size was too small to report.

Conclusions: Our analysis of a national QC data set of donors between ages 17-23 suggests that BMI has an effect on hemolysis level in male but not in female donors. A high plasma triglyceride level could be a contributor to the high level of storage hemolysis observed in male repeat high hemolyzer donors. Studies on the underlying mechanisms and the role of sex must be performed.

Katherine Serrano

CLINICAL ASSISTANT PROFESSOR

RISK ANALYSIS OF TRANSFUSION OF CRYOPRECIPITATE WITHOUT CONSIDERATION OF ABO GROUP

ABSTRACT #74

Background/objectives: Transfusion medicine standards in Canada state that adult recipients can be transfused with cryoprecipitate of any ABO blood group. However, despite current high demand for group AB cryoprecipitate, a national hospital practice survey showed that around 14% of hospitals are not willing to use cryoprecipitate of any blood group for adult transfusion. This study investigates anti-A/anti-B antibody activity in cryoprecipitate to address the safety of its transfusion without ABO matching.

Methods: To study antibody partitioning, 7 units of group O plasma were obtained from a routine component production centre. A 3.5 mL sample was taken from each unit before freezing. Cryoprecipitate was then produced from these units following standard operating procedure. Antibody titration was performed on plasma samples, cryosupernatant plasma, and cryoprecipitate using the manual tube method. IgG/IgM levels were determined by nephelometry. Additionally, 10 cryoprecipitate each from groups A, B, and O were similarly assessed. From the antibody titre distribution among these samples, the probability of making a pool of cryoprecipitate with a titer ≥1:100 was calculated using bootstrap analysis.

Results: Anti-A/B titres in cryoprecipitate were equivalent to those in corresponding plasma; partitioning of anti-A/B activity into cryoprecipitate was not observed. Average IgM concentration was higher in cryoprecipitate than in plasma ($p < 0.01$). However, no correlation between IgM levels and anti-A/B titres was established. Among 30 cryoprecipitates from routine blood bank inventory, the median antibody titre and mode were 1:32 and 1:16, respectively. Of the samples tested, 4 of 30 and 9 of 30 had titres above 1:100 and 1:50 respectively. The probability of transfusing an adult dose of cryoprecipitate (pool of 10 cryoprecipitate) with a titre higher than 1:100 was calculated to be less than 1 in 3 million.

Conclusions: This study provides strong evidence to support current transfusion medicine standards on the safety of transfusion of cryoprecipitate without the need for blood group matching in adult recipients.

Supervisor: Dr. Dana Devine

Katherine Serrano[1,2], Narges Hadjesfandiari[1,2], Elena Levin[1,2], Qi-Long Yi[2], Dana Devine[1,2]

Affiliations:
[1]Centre for Blood Research, University of British Columbia,
[2]Centre for Innovation, Canadian Blood Services

Ryan Penny

STAFF

ABSTRACT #75

Supervisor:

Ghada N Al-Rawahi[1,2], Ryan Penny[1], Audrey Cuarte[1], Suk Dhaliwal[1], Tammy Chen[1], Dale Purych[3,2], David Boyd[4], Linda Hoang[5,2], Peter Tilley[1,2]

Affiliations:
[1]BC Children's Hospital, Vancouver, BC, Canada. [2]University of British Columbia, Vancouver, BC, Canada. [3]Fraser Health, Surrey, BC, Canada. [4]Public Health Agency of Canada, Winnipeg, MB, Canada. [5]Public Health Microbiology and Reference Laboratory, Vancouver, BC, Canada

EVALUATION OF THE NG-TEST CARBA 5 MULTIPLEX IMMUNOCHROMATOGRAPHIC ASSAY AND CEPHEID XPERT CARBA-R ASSAY FOR THE DETECTION OF CARBAPENEMASE GENES IN GRAM NEGATIVE ORGANISMS

Background/objectives: The aim of this study was to evaluate the performance of the NG-Test Carba 5 multiplex immunochromatographic assay (NG Biotech, France) and the Cepheid Xpert Carba-R assay (Cepheid, Sunnyvale, CA, USA) for the detection of carbapenemase genes from pure bacterial culture. Handling time, ease of implementation, sensitivity and specificity were evaluated.

Methods: A total of 60 molecularly characterized carbapenem resistant gram negative isolates that were resistant to at least one carbapenem were included in this evaluation: 50 carbapenemase positive and 10 carbapenemase negative isolates. The isolates included are members of the order Enterobacterales (N=43), Pseudomonas aeruginosa (N=15) and Acinetobacter baumannii (N=2). Each isolate was run on both kits on the same day. NG-Test Carba 5: Visual immunoassay that uses a lateral flow device for the detection of KPC, OXA, VIM, IMP, and NDM carbapenemases. Pure isolate is combined and mixed with provided extraction buffer then applied to the testing device. A 15 minute incubation and device is read visually. Cepheid Xpert Carba-R: Cartridge-based automated real-time PCR for the detection of KPC, NDM, OXA, and IMP carbapenemases. A 0.5 McFarland of the isolate is prepared in-house of which 10uL is vortexed with the provided reagent. The filled Carba-R cartridge is then scanned and loaded into the GeneXpert testing unit. A results report is generated approximately 45 minutes later.

Results: The concordance with the reference method was 88.3% (53/60) and 85% (51/60) for the NG-Test Carba 5 and Xpert Carba-R respectively. Two false positive NDM's with faint bands were seen with the NG-Test Carba 5 which tested negative upon repeat. Almost all of the false negative results in both assays were seen with the IMP type. The overall sensitivity and specificity of the NG-Test Carba 5 and Xpert Carba-R were 89.6%, 83.3% and 82%, 100% respectively.

Conclusions: The NG-Test Carba 5 and Xpert Carba-R assays provided rapid results and are easy to implement in the clinical laboratory. The NG-Test Carba 5 assay performed slightly better for IMP type detection compared to Xpert Carba-R. The visual interpretation of NG-Test Carba 5 could be subjective so repeating is recommended when faint bands occur to prevent false positives.

Michael Lizardo

STAFF (SELECTED FOR ORAL PRESENTATION)

ABSTRACT #76

THE ROCAGLATE EUKARYOTIC INITIATION FACTOR 4A-1/2 INHIBITOR CR-1-31B HAS BOTH ANTI-TUMOUR AND ANTI-METASTATIC ACTIVITY IN OSTEOSARCOMA

Background/objectives: Osteosarcoma (OS) is a malignant primary tumor of bone where about 800 cases are diagnosed in children and young adults in the United States every year. Unfortunately treatment options for patients with metastatic disease have not improved outcomes in 3 decades. This underscores the need to develop new therapeutics that target the metastatic process. The objective of the following research is to assess the single agent activity of the eukaryotic initiation factor 4A (eIF4A)1/2 inhibitor CR-1-31B in preclinical metastatic OS models.

Methods: The IC50 values of CR-1-31B were determined in several metastatic OS cell lines including: human MG63.3, MNNG, OS PDX-derived cell line PSS008, murine F420 cells, and canine OSCA-29 cells. To assess whether mRNA translation in OS cells is modulated by CR-1-31B, OS cells were transfected with the mRNA of mCherry (a red fluorescent protein) and fluorescent microscopy was used to longitudinally observe the expression of mCherry in OS cells +/- CR-1-31B. To test whether CR-1-31B can sensitize proliferating OS cells to oxidative stress conditions, OS cells were exposed to tert-butylhydroxyquinone (tBHQ), a chemical inducer of oxidative stress. OS cells were incubated with either DMSO, +/- tBHQ, +/- CR-1-31B. Since nuclear factor erythroid 2-related factor 2 (NRF2) is a key transcription factor in the antioxidant response, protein levels of NRF2 was assessed in OS cells +/- CR-1-31B. Quantitative PCR was used to assess whether NRF2 transcript levels were affected by CR-1-31B. The ex vivo pulmonary metastasis assay (PuMA) was used to test whether CR-1-31B can inhibit the growth of OS cells in lung tissue. Anti-tumour and anti-metastatic activity of CR-1-31B was assessed in vivo using the primary leg tumour model and tail-vein injection model of metastasis, respectively.

Results: CR-1-31B had potent in vitro tumoricidal activity in all the OS models tested, with IC50 values ranging in the low nanomolar range (2-8 nM). CR-1-31B inhibited the translation of mCherry mRNA compared to DMSO control in OS cells. CR-1-31B was able to inhibit the proliferation of 4 different OS cell lines in the presence of tBHQ; whereas OS cells were able to proliferate in single agent controls. CR-1-31B blocked NRF2 upregulation in MG63.3 and MNNG cells in a dose-dependent manner. This effect was not transcriptional as NRF2 mRNA levels did not decrease with increasing CR-1-31B concentration. CR-1-31B inhibited the growth of MG63.3 and MNNG cells in murine lung tissue in the ex vivo PuMA model at 2nM compared to DMSO treatment. CR-1-31B (0.2mg/kg) significantly inhibited MG63.3 primary tumour growth and increased median survival times compared to the vehicle control group. Moreover, CR-1-31B significantly inhibited lung metastasis versus vehicle group.

Conclusions: The use CR-1-31B to target mRNA translation in OS represents a completely novel strategy for OS. Moreover, probing potential links between mRNA translation and regulation of the biological processes that underpin the metastatic process (i.e. NRF2 expression) in this disease represents a new avenue of OS research.

Supervisor: Dr. Poul Sorensen

Michael M Lizardo[1] , Yue Zhou Huang[1], Christopher Hughes[1], Sofya Langman[1], and Poul H Sorensen[1,2]

Affiliations:
[1]Department of Molecular Oncology, BC Cancer, part of the Provincial Health Services Authority. [2]Department of Pathology & Laboratory Medicine, Faculty of Medicine, University of British Columbia