





Dr. Steven Narod

PathDay: Keynote Speaker (4:30 pm)

This year's James Hogg Keynote Lecture will be given by
Dr. Steven Narod, M.D., FRCPC, Canada Research Chair
in Breast Cancer, Women's College Research Institute,
Dalla Lana School of Public Health

**Title: "Update on Hereditary Breast Cancer:
Prevention and Treatment"**

Dr. Steven Narod is the director of the Familial Breast Cancer Research Unit at WCRI and a Tier I Canada Research Chair in Breast Cancer. He is a professor in the department of public health sciences at the University of Toronto. Steven Narod is the most cited scientist in the world in the field of breast cancer. The team of scientists led by Dr. Narod includes Dr. Kelly Metcalfe and Dr. Joanne Kotsopoulos.

Research and Academic Interests:

Dr. Narod conducts longitudinal studies of women from families with and without genetic mutations related to breast cancer. He is currently focused

on translating our emerging knowledge about hereditary cancer into more effective strategies for the prevention and management of breast and ovarian cancer. He is also interested in delineating the gene/environment interactions that underlie hereditary breast cancer. This work may eventually be used to identify potential modifiers of cancer risk in high-risk groups. He is currently principal investigator on a number of studies looking at risk factors associated with hereditary breast and ovarian cancer, investigating the role of BRCA2 mutations in ovarian cancer, and investigating the contributions of CHK2 gene mutations to breast cancer risk. He also participates as a co-investigator on a wide-range of hereditary cancer studies conducted by his students and international colleagues.

WELCOME TO THE PATHOLOGY DAY 2010



Pathology Day is a critically significant event in the departmental calendar as it serves as a time to showcase research and scholarly activities performed by our trainees and, by extension, our faculty. This gathering also provides a perfect venue to recognize and acknowledge the many outstanding contributions by members of the department over the past year. Notably, Pathology Day serves another critically important function. Specifically, it is an opportunity to get together to socialize and learn about the breadth of the research and scholarly activities that take place in our geographically dispersed department. We are very fortunate to have Dr. Steven Narod (University of Toronto) as the Keynote Speaker this year that not only continues the tradition of having world leaders in biomedical research speak at Pathology Day but also highlights the foundational role of pathology and laboratory medicine in the evolution toward personalized patient-centric care. Special thanks to H el ene C ot e and Susana Martin for their efforts in organizing this year's event.

Hoping you enjoy the Day,
Michael F. Allard, Acting Head



Acknowledgment

Pathology Day is always a team effort and I would like to extend special thanks to all those who contributed to the 2010 edition. These include Dr. Mike Allard, Maureen Barfoot and Susana Martin for their help organizing this event. A special thanks to Debbie Bertanjoli for the preparation of the abstract book. I also wish to extend my heartfelt gratitude to the many people who contributed their time and expertise for the reviewing of abstract, moderating of the oral sessions and judging for the oral and poster presentation awards. This year, these include Amanda Bradley, Cedric Carter, Blake Gilks, David Granville, John Hill, David Huntsman, Jay Kizhakkedathu, Wan Lam, Jacque Quandt, Mike Nimmo, Catherine Pallen, Susan Porter, Haydn Pritchard, Ed Pryzdial, Marianne Sadar, Chun Seow, Bruce Verchere, David Walker, Rebecca Walters and Cheryl Wellington. Finally, sincere thanks to the department's staff and volunteer students who assist with various tasks and contribute to the day's success.

H el ene C ot e
Chair Pathology Day 2010

Special
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Conference Outline

Pathology Day 2010

08:30 Breakfast - Plaza 500 Hotel 500 West 12th Avenue, Vancouver, BC

Resident Oral Session, Granville Room

- abstract# 09:00 Opening Remarks by Dr. Michael Nimmo
- 1 09:15 **Arwa Al-Riyami**
Octaplex usage one year after implementation at a tertiary care hospital
- 2 09:30 **Ahmad Al-Sarraf**
Central pontine myelinolysis following orthotopic liver transplantation- a rare complication
- 3 09:45 **Titus Wong**
Evaluation of real-time PCR tcdC gene detection assay for the diagnosis of *Clostridium difficile* infection
- 4 10:00 **Elaine Willman cancelled**
A prospective case series of single-syringe ketamine-propofol combination (ketofol) for emergency department procedural sedation and analgesia
- 10:15 - 10:45 BREAK
- 5 10:45 **Anna Lee**
Periodic acid-schiff is superior to hematoxylin & eosin for screening prophylactic gastrectomies from CDH1 mutation carriers.
- 6 11:00 **Fang-I Lu**
Prevalence of DNA mismatch repair gene defects amongst malignant ovarian tumors: an immunophenotypic analysis
- 7 11:15 **Dmitry Turbin**
Hematoxylin counterstaining influences visual assessment of immunostained FFPE slides
- 8 11:30 **Vincent Fung**
Evaluation of circulating tumor cells as prognostic markers in patients with metastatic renal cell carcinoma (mRCC) receiving systemic therapy
- 11:45 - 13:15 LUNCH
- 13:15 - 15:15 POSTER PRESENTATIONS
- 9 15:15 **David F. Schaeffer**
Absolute increase in endocrine cells in pediatric gastric biopsies following long-term proton pump inhibitor therapy
- 10 15:30 **Tony Ng**
Identification and characterization of a novel TCF12-LIN28B fusion in sporadic Wilms' tumor
- 11 15:45 **Majid Zolein**
A performance audit of adrenal vein sampling in British Columbia: how are we doing?

Graduate Students and Post-Doctoral Fellows Oral Session, Oak Room

- abstract # 09:00 Opening Remarks by Dr. Jacqueline Quandt
- 12 09:15 **Clara Westwell-Roper**
Macrophage recruitment and TNF-alpha release: a pro-inflammatory role for human islet amyloid polypeptide
- 13 09:30 **Ashish Marwaha**
Bias towards il-17 secreting t cells in children with new onset type 1 diabetes

Conference Outline

2010

abstract #

- 14 09:45 **Rachel Wade**
Ishak-knodell pathology score, mitochondrial DNA content and mitochondrial gene expression in liver from patients co-infected with HIV and hepatitis C virus
- 15 10:00 **Amanda Vanden Hoek**
Identification of a novel coagulation factor X compound heterozygous mutation associated with moderate bleeding
- 10:15 - 10:45 BREAK
- 16 10:45 **Farshid S. Garmaroudi**
Deconstruction of the coxsackievirus B3-induced signaling network in cardiomyocytes
- 17 11:00 **Xin Ye**
microRNA(mir)-126 promotes coxsackievirus B3 replication by enhancing ERK signaling pathway
- 18 11:15 **Wendy Boivin**
Granzyme B cleaves proteoglycans and releases sequestered TGF- β from extracellular matrix: implications in atherosclerosis and plaque instability
- 19 11:30 **Lisa Ang**
Serpine A3N reduces abdominal aortic aneurysm rupture in mice by inhibition of extracellular granzyme B
- 11:45 - 13:15 LUNCH
- 13:15 - 15:15 POSTER PRESENTATIONS
- 20 15:15 **Kevin (Yu Chi) Yang**
Novel small molecules that inhibit androgen receptor have effect on tumor proliferation in prostate cancer
- 21 15:30 **Jennifer Kennett**
Characterization of genomic alterations in a subset of retinoblastoma tumors using high resolution array comparative genomic hybridization
- 22 15:45 **Brian K. Chung**
Natural killer T cells kill Epstein-Barr virus transformed B cells following CD1d upregulation by retinoic acid receptor-alpha
- 23 16:00 **Cristina Tognon**
Control of Oncogenic Fusion Protein Levels by the Insulin-like Growth Factor Receptor-1 pathway

James Hogg Keynote Lecture, Granville/Oak Room

- 16:30 **Dr. Steven Narod, M.D., FRCPC**, Canada Research Chair in Breast Cancer, Women's College Research Institute, Dalla Lana School of Public Health
"Update on Hereditary Breast Cancer: Prevention and Treatment"

Reception, Dinner & Awards Presentations, Vancouver Art Gallery

- 18:00 750 Hornby Street
Vancouver, BC V6Z 2H7 (604) 662-4700

Table of Content

Oral Presentations

abstract #

1	Octaplex usage one year after implementation at a tertiary care hospital	11
	<i>Arwa Al-Riyami¹, Kristine Roland^{1,2}, Kate Chipperfield^{1,2}</i>	
2	Central pontine myelinolysis following orthotopic liver transplantation- a rare complication	12
	<i>Ahmad J. Al-Sarraf MD¹, Mazhar Haque MB BS, FRACP², Morris Pudek BSc, PhD³, Eric M. Yoshida, MD, MHSc, FRCP(C)²</i>	
3	Evaluation of real-time PCR tcdC gene detection assay for the diagnosis of <i>Clostridium difficile</i> infection	13
	<i>Titus Wong, Marc Romney, Gordon Ritchie, Sue Pengilly, and Sylvie Champagne</i>	
4	A prospective case series of single-syringe ketamine-propofol combination (ketofol) for emergency department procedural sedation and analgesia	14
	<i>Elaine Willman¹, Gary Andolfatto²</i>	
5	Periodic acid-Schiff is superior to hematoxylin & eosin for screening prophylactic gastrectomies from CDH1 mutation carriers	15
	<i>Anna F Lee, MD CM PhD¹ Henrike Rees, MD FRCPC², David A Owen, MB BCH FRCPC¹, David G Huntsman, MD FRCPC^{1,3}</i>	
6	Prevalence of DNA mismatch repair gene defects amongst malignant ovarian tumors: an immunophenotypic analysis	16
	<i>Fang-I Lu¹, C. Blake Gilks¹, Paul Ryan², Blaise Clarke³</i>	
7	Hematoxylin counterstaining influences visual assessment of immunostained FFPE slides.	17
	<i>D. Turbin¹, D. Gao², J. Garratt¹, R. Wolber¹, S. Leung², T. Nielsen^{1,2}, B. Gilks^{1,2}</i>	
8	Evaluation of circulating tumor cells as prognostic markers in patients with metastatic renal cell carcinoma (mRCC) receiving systemic therapy	18
	<i>Vincent Fung¹, Jenny Baybik², Kim Chi³</i>	
9	Absolute increase in endocrine cells in pediatric gastric biopsies following long-term proton pump inhibitor therapy	19
	<i>David F Schaeffer¹, Eric Hassal², Teresa Sturby¹ and David A Owen¹</i>	
10	Identification and characterization of a novel TCF12-LIN28B fusion in sporadic Wilms' tumor	20
	<i>Tony Ng^{1,2}, Matthew Robertson¹, Hao Zhu³, William Einhorn³, George Daley³, Poul Sorensen^{1,2}</i>	
11	A performance audit of adrenal vein sampling in British Columbia: how are we doing?	21
	<i>Majid Zolein¹, Daniel T. Holmes^{1,2}</i>	
12	Macrophage recruitment and TNF-alpha release: a pro-inflammatory role for human islet amyloid polypeptide	22
	<i>Clara Y. Westwell-Roper¹, Derek L. Dai¹, Kathryn J. Potter¹, C. Bruce Verchere^{1,2}</i>	
13	Bias towards il-17 secreting t cells in children with new onset type 1 diabetes	23
	<i>Ashish K. Marwaha¹, Constadina Panagiotopoulos², John J. Prialat¹, Megan K. Levings³ & Rusung Tan¹</i>	
14	Ishak-knodell pathology score, mitochondrial dna content and mitochondrial gene expression in liver from patients co-infected with hiv and hepatitis c virus.	24
	<i>Rachel E Wade¹, Mark Hull², Valentina Montessori², Julio Montaner², Marianne Harris², Marissa Jitratkosol¹, Isabelle Gadawski¹, H�el�ene C�ot�e¹</i>	
15	Identification of a novel coagulation factor x compound heterozygous mutation associated with moderate bleeding.	25
	<i>Amanda Vanden Hoek^{1,2,3}, Kim Talbot^{1,2}, Isis Carter^{1,2,4}, Linda Vickars⁵, Ross T.A. MacGillivray^{2,6}, Edward L.G. Prydzial^{1,2,3}</i>	
16	Deconstruction of the coxsackievirus b3-induced signaling network in cardiomyocytes.	26
	<i>Farshid S. Garmaroudi¹, David Marchant¹, Ali Bashashati², Raymond T. Ng^{1,3}, Kevin P. Murphy^{3,4}, Honglin Luo¹, Kevin A. Janes⁵ and Bruce M. McManus¹</i>	
17	microRNA(mir)-126 promotes coxsackievirus B3 replication by enhancing ERK signaling pathway.	27
	<i>Xin Ye, Mary Zhang, Zhen Liu, Maged Hemida, Decheng Yang</i>	
18	Granzyme B cleaves proteoglycans and releases sequestered TGF-� from extracellularmatrix: implications in atherosclerosis and plaque instability.	28
	<i>Wendy A. Boivin^{1,2}, Marlo Shackelford², Hongyan Zhao², and David J. Granville^{1,2}</i>	
19	Serpin A3N reduces abdominal aortic aneurysm rupture in mice by inhibition of extracellular granzyme B.	29
	<i>Lisa S. Ang¹, Sarah J. Williams¹, Wendy A. Boivin¹, Hongyan Zhao¹, Tyler Varnals¹, Bruce M. McManus¹, Michael F. Allard¹, R. Chris Bleackley², David J. Granville¹</i>	

Table of Content

20	Novel small molecules that inhibit androgen receptor have effect on tumor proliferation in prostate cancer	30
	<i>Yu Chi Yang¹, Nasrin (Rina) Mawji¹, David E Williams², Raymond Andersen², and Marianne D Sadar¹</i>	
21	Characterization of genomic alterations in a subset of retinoblastoma tumors using high resolution array comparative genomic hybridization	31
	<i>Jennifer Y. Kennett^{1,2}, Stephanie Yee³, Wan L. Lam^{1,2}</i>	
22	Natural killer T cells kill epstein-barr virus transformed B cells following CD1d upregulation by retinoic acid receptor-alpha	32
	<i>Brian K. Chung, Lenka L. Allan, John J. Priatel, Peter van den Elzen and Rusung Tan</i>	
23	Control of oncogenic fusion protein levels by the insulin-like growth factor receptor-1 pathway	33
	<i>C.E. Tognon¹, G. Trigo¹, B. Rotblat¹, PHB Sorensen¹</i>	

Poster Presentations

abstract #

24	Hace1 E3 ligase tumor suppressor regulates proteasome activity	35
	<i>Naniye Cetinbas^{1,2}, Barak Rotblat^{1,2}, Thibault Mayor³, and Poul HB Sorensen^{1,2}</i>	
25	Harvesting value from human tissues at the time of cardiac transplantation: one story from the cardiovascular biobank	36
	<i>Crystal Leung, Lise Matzke, Mike Allard, Bruce McManus</i>	
26	Methodological issues related to pathological evaluation of coronary artery stents	37
	<i>Crystal Leung, Amrit Samra, Lise Matzke, Mike Allard</i>	
27	Fatty and fibrofatty phenotypes of arrhythmogenic right ventricular cardiomyopathy in sudden death and heart failure: immunohistochemical analysis of N-cadherin and plakoglobin	38
	<i>Lise Matzke¹, W. Mark Elliott¹, Crystal Leung¹, Carol Lee², Charles Lee², Bruce McManus¹, Mike Allard¹</i>	
28	Cardiac amyloid in the setting of transcatheter valve implantation: a possible contributor of post-operative death?	39
	<i>Lise Matzke, Fabian Nietlispach, John Webb, Sam Lichtenstein, Michael Allard</i>	
29	Modulation of cardiac metabolism by beta-blockers during diabetes: a role in apoptosis signaling	40
	<i>Varun V. Saran¹, Vijay Sharma², Violet G. Yuen³ Rich Wambolt¹, Michael F. Allard¹ and John H. McNeill³</i>	
30	Gene and protein expression alterations in the myocardium following mechanical circulatory assist in heart failure patients.	41
	<i>Anna Meredith, Amrit Samra, Lise Matzke, Crystal Leung and Bruce McManus</i>	
31	Lipid-based formulation of irinotecan (Irinophore C), vincristine and doxorubicin target tumor vasculature in glioblastoma multiforme	42
	<i>Maite Verreault^{1,3}, Dita Strutt¹, Dana Masin¹, Malathi Anantha¹, Dawn Waterhouse¹, Marcel B Bally^{1,2,3,4}, Don T Yapp^{1,2}</i>	
32	Suppression of Her2/neu expression through ILK inhibition is regulated by a pathway involving TWIST and YB-1	43
	<i>Jessica Kalra^{1,3}, Brent W. Sutherland¹, Anna L. Stratford⁷, Karen A. Gelmon¹, Shoukat Dedhar², Sandra E. Dunn^{4,7}, and Marcel B. Bally^{1,5,6,8}</i>	
33	Stavudine induces insulin resistance in cultured adipocytes through alteration in adipokine secretion and activation of AMP-activated protein kinase	44
	<i>Ramesh Saeedi¹, Varun Saran¹, Kevin Johns¹, Marianne Harris², Julio Montaner^{1,3}, Michael Allard¹, Greg Bondy¹</i>	
34	Developing a method for assessing the effect of anti-retroviral HIV therapy on placental defence mechanisms	45
	<i>Alisa Abozina¹, Eszter Papp, Hélène Côté</i>	
35	Liver ultrastructural damage in hepatitis C virus and HIV co-infected individuals	46
	<i>Hayley Spencer^{1,2}, Rachel Wade², Fanny Chu¹, Hélène Côté² and David Walker^{1,2}</i>	
36	Peripheral blood telomere length in infants and their HIV-infected mothers treated with antiretroviral therapy during pregnancy	47
	<i>Tuhina Imam¹, Hugo Soudeyns⁴, Normand Lapointe⁴, Marissa Jitratkosol¹, Beheroze Sattha¹, Johanne Samson⁴, Tessa Chaworth-Musters³, John Forbes^{2,3}, Deborah Money^{1,2} and Hélène Côté^{4*1,2}</i>	

Table of Content

abstract

37	Expression of a multidrug-resistance protein (p-glycoprotein) gene (MDR-1) in the placenta of HIV infected and HAART-Exposed Women	48
	<i>Eszter Papp, Deborah Money, Laura Oliveira, Tuhina Imam, Beheroze Sattha, Izabella Gadawski, Evelyn Maan, Jack Forbes, H��l��ne C��t��</i>	
38	Cardiolipotoxicity and hepatic dysfunction is augmented in Cbs +/- mice with diet-induced obesity	49
	<i>Melissa B Glier¹, Dian C Sulistyoningrum², Sanjoy Gosh¹, and Angela M Devlin^{1,2}</i>	
39	Promoting pathology and laboratory medicine to medical students: The UBC Pathology and Laboratory Medicine Student Interest Group (PaLM-SIG)	50
	<i>Serena Siow, Jacalynne Hernandez-Lee</i>	
40	Characterization of patients with very high plasma HDL-cholesterol, with and without vascular pathology	51
	<i>Ahmad Al-Sarraf MD^{1,2}, Gautamn Sarwal, BMLSc^{2,3}, Catalin Taraboanta, MD MSc¹, and Jiri Frohlich, MD FRCP(C)^{1,2}</i>	
41	Deregulation of oncogene and tumor suppressor expression by methylation in early stage oral c	52
	<i>Shevaun E. Hughes¹, Ivy F.L. Tsui¹, Catherine Poh^{2,3}, and Cathie Garnis^{1,2}</i>	
42	Molecular characterization of anaplastic thyroid carcinomas	53
	<i>Rebecca Towle¹, Danielle MacNeill¹, Ken Berean², Don Anderson¹, Cathie Garnis¹</i>	
43	Is the third AFB smear necessary to remove patients from respiratory isolation.	54
	<i>Amanda Wilmer^{1,2}, Elizabeth Bryce^{1,2}, Jennifer Grant^{1,2}</i>	
44	Skin aging as an indicator of cardiovascular health in apolipoprotein E knockout mice	55
	<i>Paul R. Hiebert^{1,2}, Thomas Abraham¹, Sara Pazooki¹, Wendy A. Boivin^{1,2}, Hongyan Zhao¹, David J. Granville^{1,2}</i>	
45	DNA quantity and quality from paraffin blocks: a comparison of fixation, processing and nucleic acid extraction techniques.	56
	<i>Gulisa Turashvili¹, Melinda Carrier¹, Nadia Gale¹, Ying Ng¹, Katie Chow¹, Lynda Bell¹, Margaret Luk², Steve Kalloger¹, Blake Gilks², Samuel Aparicio¹, David Huntsman¹</i>	
46	Microbial sequence characterization of ovarian cancer tissue	57
	<i>V. Montoya¹, J. Gardy^{1,2}, K. Jewell¹, S. Shah^{1,3}, D. Huntsman^{1,3}, P. Tang^{1,2}</i>	
47	20(S)-protopanaxatriol affects the expression of cytochrome P450 3A4	58
	<i>Nga Ting Colette Chiu¹, Emma Tomlinson Guns², William Jia³</i>	
48	Death by boric acid - was Surekiller ^{��} a sure killer?	59
	<i>Walter Martz and Henry Kaliciak</i>	
49	Combination of high performance liquid chromatography fluorescence detection and modern mass spectrometry for analysis of primary amine derivatives in biological fluids and tissues	60
	<i>Josephine H. Cheng¹, Christine Joseph¹, and Bernd O. Keller¹</i>	
50	Protein targets regulated by polyunsaturated fatty acid maternal diet in fetal rat brain.	61
	<i>Katelyn J. Janzen¹, Elizabeth M. Novak², Sheila M. Innis², and Bernd O. Keller¹</i>	
51	Short antimicrobial peptides immobilized copolymer brushes on titanium surface.	62
	<i>Guangzheng Gao, Jason Kindrachuk, Donald E. Brooks, Robert E.W. Hancock, and Jayachandran N. Kizhakkedathu</i>	
52	Macromolecular iron chelators: a novel therapeutic agent for the treatment of secondary iron overload	63
	<i>Jasmine L. Hamilton, Muhammed Imran ul-haq, Iren Constantinescu, and Jayachandran N. Kizhakkedathu</i>	
53	Synthesis of biomimicking glycopolymer brushes containing different carbohydrate residues and their non-biofouling properties	64
	<i>Kai Yu, Jayachandran N Kizhakkedathu</i>	
54	A novel lung tumor suppressor implicated in somatic and familial cancers	65
	<i>Ian M. Wilson¹, Katey S. S. Enfield¹, Emily A. Vucic¹, Raj Chari¹, Yu-An Zhang², Ming You³, Calum MacAulay¹, Stephen Lam¹, Adi F. Gazdar², Wan L. Lam¹</i>	
55	Amplification status of GAS41 and MDM2 in lung adenocarcinomas	66
	<i>Larissa Pikor¹, William W. Lockwood², Raj Chari¹, Emily A. Vucic¹, Stephen Lam¹, Adi F. Gazdar³ and Wan L. Lam¹</i>	
56	Frequent DNA deletion of miRNA-101, a candidate tumour suppressor, in lung cancer.	67
	<i>Kelsie L. Thu^{1,3}, Raj Chari^{2,3}, Wan L. Lam^{2,3}</i>	

Table of Content

abstract

57	The role of eukaryotic translation initiation factor 2 alpha related pathways in autophagosome formation during Cocksackievirus B3 induced myocarditis	68
	<i>Tak Kwong Poon¹, Jerry Wong¹, John Zhang¹, Guang Gao¹, Honglin Luo¹</i>	
58	Avitene-induced necrotizing granulomatous inflammation developing post-craniotomy: a pediatric case series	69
	<i>Liat Apel-Sarid¹, Doug Cochrane², Paul Steinbok², Angela Byrne³, Christopher Dunham¹</i>	
59	University of British Columbia microbiology curriculum Improvement report	70
	<i>Kristel Lobo Prabhu¹, Niamh Kelly¹, Linda Hoang¹, Muhammad Morshed¹</i>	
60	Using tissue microarrays to develop an immunohistochemical panel for basal-like breast cancer	71
	<i>Jennifer R. Choo^{1,2}, Samuel Leung², Dongxia Gao², Christine Chow², Sherman Y.H. Lau², Jinjin Cheng² and Torsten O. Nielsen^{1,2}</i>	
61	Integrin-induced protein tyrosine phosphatase alpha (PTPa) tyrosine phosphorylation regulates cas-mediated cell migration.	72
	<i>Suzanne Cheng^{1,4}, Guobin Sun^{2,4}, Catherine J. Pallen^{1,2,3,4}</i>	
62	Herpesviruses enhance generation of the clot-dissolving enzyme, plasmin, by tissue plasminogen activator: Correlation to virus-surface annexin II	73
	<i>Edwin S. Gershom¹, Michael R. Sutherland^{1,2} and Edward L. G. Pryzdial^{1,2}</i>	
63	Phenotypic and genomic variability of the 1q21.1 copy number variant (CNV) region in multigenerational families... 74	
	<i>*Harvard C.^{1,3}, *Strong E.^{1,3}, Martell S.^{1,3}, Tyson C.⁶, McGillivray B.⁴, Hamilton S.⁴, Marles S.⁷, Mhanni A.⁷, Chow E.¹⁰, Pavlidis P.⁵, Qiao Y.^{1,4}, Holden J.J. A.^{8,9}, Lewis M.E. S.^{1,4}, and Rajcan-Separovic, E.^{1,4} (*1st & 2nd author contributed equally to this work)</i>	
64	Outcome of array CGH analysis for 255 subjects with intellectual disability (ID) and search for candidate genes using bioinformatics	75
	<i>Ying Qiao^{1,2}, Chansonette Harvard¹, Christine Tyson³, Xudong Liu⁴, Celina Fawcett³, Paul Pavlidis⁵, Jeanette J.A. Holden⁴, Suzanne M.E. Lewis², Evica Rajcan-Separovic¹</i>	
65	Effect of iron on antigen presenting cells: implications for transfusion dependent hemoglobinopathies.	76
	<i>Ibrahim Mustafa, Duncheng Wang and Mark D. Scott</i>	
66	Mechanical properties of asthmatic airway smooth muscle revisited.	77
	<i>Leslie YM Chin^{1,4}, Ynuk Bossé⁴, Dennis Solomon⁴, Tillie L Hackett^{3,4}, Peter D. Paré^{2,4}, and Chun Y. Seow^{1,4}</i>	
67	Y box binding protein-1 is a major contributor to sarcoma cells motility and aggressiveness.	78
	<i>Amal M. El-Naggar^{1,2}, Cristina Tognon², Fan Zhang², Joan Mathers², and Poul HB. Sorensen^{1,2}</i>	
68	The activation of the phosphatidylinositol-3 kinase pathway in response to the burkholderia cepacia complex infection	79
	<i>Billie Velapatiño¹ and David P Speert²</i>	
69	Evaluation of two chromogenic media for screening of group b streptococcus in pregnant women	80
	<i>Laura Book¹, Ghada N. Al-Rawah^{1,2}, Eva Thomas^{1,2}</i>	
70	The role of brain high density lipoproteins in facilitating beta-amyloid degradation.	81
	<i>Sophie Stukas¹, Sharon May¹, Braydon Burgess², Nicole DeValle², Anna Wilkinson¹, Veronica Hirsch-Reinshagen¹, Mandie Baker², Michael N. Oda² and Cheryl L. Wellington¹</i>	
71	Cocksackievirus B3-induced downregulation of p58ipk is involved in shifting endoplasmic reticulum stress to cell apoptosis	82
	<i>Mary Zhang, Xin Ye, Goma MH Hemida, Stefanie Mak and Decheng Yang</i>	
72	Effect of polymer species and size on immunocamouflage and antigenicity	83
	<i>Kyluik DL and Scott MD</i>	
73	Regulation of cd1d expression in human b cells by retinoic acid receptor	84
	<i>Lenka L. Allan, Annelein M. Stax, Dong-Jun Zheng, Brian K. Chung, Rusung Tan, and Peter van den Elzen</i>	
74	T regulatory cell mediated protection of allograft in type 1 diabetes.	85
	<i>Joel Montane¹, Loraine Bischoff¹, Galina Soukhatcheva¹, Lei Dai¹, Gijs, Hardenberg², Megan K. Levings², Rusung Tan¹, C. Bruce Verchere^{1,2}</i>	
75	Sulfatide-reactive T cells in multiple sclerosis	86
	<i>Annelein Stax, Lenka Allan, Dong Jun Zheng, Peter van den Elzen</i>	



ORAL PRESENTATIONS

Arwa Al-Riyami¹, Kristine Roland^{1,2}, Kate Chipperfield^{1,2}

¹Department of Pathology and Laboratory Medicine, University of British Columbia

²Department of Pathology and Laboratory Medicine, Vancouver General Hospital



Arwa Al-Riyami

Abstract # 1

OCTAPLEX USAGE ONE YEAR AFTER IMPLEMENTATION AT A TERTIARY CARE HOSPITAL

Background/Objectives

Octaplex[®] is a Human Prothrombin Complex Concentrate that was licensed by Health Canada in 2008 for the emergent management of acquired prothrombin complex deficiency (ie warfarin therapy). The product has been in use at our tertiary care hospital since September 2008. Requests for Octaplex[®] require Hematopathologist approval, and the dose is dependent on patient's weight and INR. Recent national recommendations for dosing Octaplex[®] conflict somewhat with the manufacturer's approach, resulting in Hematopathologist discretion when deciding on a dose.

This review evaluated our institution's experience with Octaplex[®] use in emergency settings. We wished to examine consistency of dosing regimens and the efficacy of doses administered.

Methods

We performed a retrospective review of all issues of Octaplex[®] from Vancouver General Hospital's blood bank from September 1st 2008 to January 31st 2010. Clinical indications and details of product administration were obtained by manual review of patient medical records. Data obtained included clinical indication and the doses administered. Pre and post treatment INR's and patient outcomes were documented.

Results

A total of 42 orders for Octaplex[®] were received between September 2008 and January 2010 inclusive. Most of the orders received were from the Emergency department (18 patients), with the most common indication being a life threatening bleed (69%). The most frequently administered dose was 1000 IU (range 500-4000). Treatment success rates at reducing the INR to <1.5 were 81% immediately post infusion, and 88% at 24 hours; excluding patients who were not tested or deceased. Seven patients failed to reverse the INR immediately post Octaplex[®], six of which did however achieve clinical hemostasis; defined by control of bleed and/or stabilization of the hemoglobin. Thromboembolism was reported in four patients, two of which developed within two days of Octaplex[®] use.

Conclusion

Octaplex[®] is increasingly being used for the emergency reversal of warfarin. It has demonstrated efficacy in INR reversal in the majority of our patients in doses of at least 1000 IU. Further studies are warranted to determine the most effective dosing strategy.



Ahmad Al-Sarraf

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CENTRAL PONTINE MYELINOLYSIS FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION- A RARE COMPLICATION

Background/Objectives

Orthotopic liver transplantation (OLT) is the treatment of choice for many acute and chronic end-stage liver diseases as well as selected cases of primary liver malignancy. With the increased number of OLT, some neurological complications have become apparent. Central pontine myelinolysis (CPM) is a rare and severe neurological complication characterized by acute central pontine neuronal demyelination along with severe occasionally irreversible manifestations. We report a patient diagnosed with CPM following OLT in the absence of significant hyponatremia. We suggest that several factors may contribute to the development of CPM following OLT.

Methods

A 46-year-old female patient underwent orthotopic liver transplantation due to end stage decompensated cryptogenic cirrhosis with hepatic encephalopathy. Post transplant period was complicated with renal impairment. Later, she developed hepatic artery thrombosis and lost her graft. However, clinically she was neurologically unremarkable. She underwent a second transplant a month after the initial one. postoperatively, she developed neurologic symptoms characteristics of CPM and MRI showed changes compatible with CPM with no dramatic changes in sodium plasma level.

Conclusion

Central pontine myelinolysis has a multifactor aetiology. Slow correction of hyponatremia is crucial for the prevention of CPM, but attention should be given to the group of patients with higher risk factors such as liver transplantations, alcoholism, and malnutrition. It should be kept in mind that CPM can affect patients with low, normal, or elevated serum levels of sodium.

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Titus Wong

Abstract # 3

EVALUATION OF REAL-TIME PCR tcdC GENE DETECTION ASSAY FOR THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE* INFECTION

Background/Objectives

The tcdC gene within the *Clostridium difficile* pathogenicity cassette is a negative regulator for toxins A and B expression. Deletions in the tcdC gene are associated with more virulent strains of *C. difficile* including ribotype 027. In this prospective study, a *C. difficile* GDH antigen enzyme-linked immunoassay (Techlab, Blacksburg, VA), a *C. difficile* Toxin A/B enzyme-linked immunoassay (Techlab, Blacksburg, VA), cell cytotoxicity assay, *C. difficile* culture and an in-house real-time PCR amplifying the tcdC gene were evaluated..

Methods

Faecal samples from hospitalized patients with a query diagnosis of *C. difficile* infection (CDI) were collected for two consecutive months at St. Paul's Hospital, Vancouver, Canada. In total, 430 faecal samples were obtained from 343 patients. Samples were first tested according to the hospital's three step "standard operating protocol" (SOP), which consists of (1) GDH antigen ELISA, (2) Toxin A/B ELISA and (3) cell cytotoxicity assay. All faecal samples were cultured on *C. difficile* agar and then frozen for further studies. Samples were then tested with an in-house tcdC gene real-time PCR for performance evaluation. Discordant results were resolved by performing an ethanol shock treatment; recovered isolates underwent toxigenic culture assay, which is considered the gold standard for statistical analysis..

Results

There were 331 concordantly-negative, 62 concordantly-positive, and 37 discordant results for a total concordance/discordance rate of 91% / 9%. After resolution of discordant results, the sensitivity for culture, SOP, and PCR were 100%, 70.4%, and 100%, respectively. The specificity for culture, SOP, and PCR were 97.6%, 99.4%, and 99.7%. The positive and negative predictive values for culture, SOP, and PCR were 91.7%/100%, 96.9%/93.0%, and 98.9%/100%. For the 37 discordant results, agreement with the gold standard for culture, SOP and PCR was 78.4%, 24.3%, and 97.3%..

Conclusion

We concluded that this in house real-time PCR for the detection of *C. difficile* tcdC gene is both sensitive and specific in our hospital setting for patients with a query diagnosis of CDI. For resolving discordant results, *C. difficile* tcdC gene real-time PCR had the highest concordance rates when compared to toxigenic culture..



Elaine Willman

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A PROSPECTIVE CASE SERIES OF SINGLE-SYRINGE KETAMINE-PROPOFOL COMBINATION (KETOFOFOL) FOR EMERGENCY DEPARTMENT PROCEDURAL SEDATION AND ANALGESIA

Background/Objectives

This study evaluated the effectiveness, recovery time, and adverse event profile of intravenous (IV) ketofol (mixed 1:1 ketamine-propofol) for emergency department (ED) procedural sedation and analgesia (PSA).

Methods

Prospective data were collected on all PSA events performed in the ED from July 2005 to December 2009 at Lion's Gate Hospital, North Vancouver - a level 3 trauma center and community teaching hospital. From this data set, all PSAs using a single-syringe 1:1 mixture of 10 mg/mL ketamine and 10 mg/mL propofol (ketofol) were analyzed. Effectiveness, recovery time, physiologic data, drug doses, adverse events, recovery time, and RN/physician satisfaction were recorded.

Results

Ketofol PSA was performed in 1014 patients with a median age of 41 years (range = 1 to 99 years; interquartile range [IQR] = 18 to 63 years). Procedures were primarily orthopedic (71%), followed by incision and drainage (11%), and cardioversion (8%). The median dose of medication administered was 0.74 mg/kg each of ketamine and propofol (range = 0.2 to 3.0 mg/kg; IQR = 0.6 to 1.0 mg/kg). Median recovery was 13 minutes (range = 1 to 50 minutes; IQR = 10 to 17 minutes).

Ketofol PSA was effective in 1004 cases (99%). Apnea occurred in 20 patients (1.9%; 95% confidence interval [CI] = 1.1% to 2.7%), of which 16 required bag-mask ventilation (1.5%; CI = 0.8% to 2.3%). Recovery agitation requiring treatment occurred in 17 patients (1.6%; CI = 0.8% to 2.4%). Transient hypertension occurred in 67 patients (6.6%; CI = 5.1% to 8.1%), of whom 2 received treatment. Median RN and physician satisfaction ratings were 10 on a 1-to-10 scale.

Conclusion

Ketofol PSA is a highly effective ED PSA regimen in patients of all ages. Recovery times were short; adverse events were few; and staff were highly satisfied.

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Anna F Lee

PERIODIC ACID-SCHIFF IS SUPERIOR TO HEMATOXYLIN & EOSIN FOR SCREENING PROPHYLACTIC GASTRECTOMIES FROM CDH1 MUTATION CARRIERS

Background/Objectives

Hereditary diffuse gastric cancer (HDGC), an autosomal dominant cancer susceptibility syndrome, is largely attributable to germline mutations and deletions in the gene encoding E-cadherin, CDH1. Asymptomatic, mutation-positive individuals often choose prophylactic gastrectomy for cancer risk reduction. Examination of the entire mucosa of prophylactic gastrectomy specimens is essential and reveals occult gastric cancers in most cases. We hypothesized that primary screening entire cases stained with periodic acid-Schiff (PAS) instead of hematoxylin and eosin (H&E) could improve diagnostic accuracy and speed of detecting invasive signet-ring adenocarcinoma.

Methods

Serial sections from six prophylactic gastrectomy specimens with molecularly confirmed CDH1 mutations were stained with PAS and H&E respectively (108-164 blocks per case). PAS- and H&E-stained slides were randomized for each case and examined microscopically for the presence of invasive signet-ring cells. The time to examine each slide was recorded.

Results

Significantly fewer lesions were missed (i.e. the lesion was initially identified on only one section, but present on both sections) on PAS-stained slides (6 missed lesions) than on H&E-stained slides (23 missed lesions); ($p < 0.05$). Furthermore, it took significantly less time to screen a PAS- stained case (3 hr 05 min +/- 41 min) than an H&E-stained case (4 hr 59 min +/- 1 hr 2 min) ($p < 0.05$). Selected lesions were confirmed as epithelial by pan-keratin-positive immunohistochemistry.

Conclusion

Doing PAS staining instead of H&E on CDH1 mutation positive prophylactic gastrectomy specimens may increase the detection rate of adenocarcinoma while reducing screening time..



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PREVALENCE OF DNA MISMATCH REPAIR GENE DEFECTS AMONGST MALIGNANT OVARIAN TUMORS: AN IMMUNOPHENOTYPIC ANALYSIS

Background/Objectives

Lynch syndrome is an autosomal dominant condition caused by germline mutation in one of the DNA mismatch repair genes (hMLH1, hMSH2, hMSH6 and PMS2), and is associated with an increased risk for multiple malignancies, with colorectal carcinoma and endometrial carcinoma being the most common. Patients with Lynch syndrome are also at an increased risk for ovarian malignancies, with a lifetime risk estimated at 10-12%. Little is known about the patient characteristics, morphologic features and prognosis of Lynch syndrome-associated ovarian malignancies. We analyzed the prevalence of DNA mismatch repair gene defects amongst ovarian malignancies, using immunohistochemical staining for DNA mismatch repair gene (MMR) products on tissue microarrays (TMA) consisting of various ovarian malignancies. We plan to correlate these findings with patient characteristics, tumor morphology and patient outcome. Finally, this study evaluates the feasibility of using TMA immunohistochemical expression for MMR gene products to assess MMR gene defects in tumors, with the potential of using this technique on other hereditary and sporadic tumors with MMR gene defects.

Methods

TMA consisting of 445 malignant ovarian tumors collected at Vancouver General Hospital (VGH) were stained for hMLH1, hMSH2, hMSH6, and hPMS2 at the Genetic Pathology Evaluation Centre (GPEC). The stained TMA slides were independently scored by investigators from VGH (FL and BG) and by investigators from Mount Sinai Hospital and Toronto General Hospital in Toronto (PR and BC). The whole-section slides of cases found to demonstrate absence of staining for any of the MMR gene products will be stained for MMR gene products. Patient characteristics, tumor morphology and patient outcome will also be assessed for cases demonstrating true absence of staining with MMR gene products.

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Dmitry Turbin

Abstract # 7

HEMATOXYLIN COUNTERSTAINING INFLUENCES VISUAL ASSESSMENT OF IMMUNOSTAINED FFPE SLIDES

Background/Objectives

Assessment of Ki67 immunostaining in formalin-fixed paraffin-embedded breast carcinoma tissues has been used since the mid 1980s. Percent of positive cells alone or in combination with the intensity of staining was examined in different studies, and has consistently been shown to be an independent prognostic factor. Recently, its role in distinguishing between luminal type A and B breast carcinoma has been evaluated. An impediment to the implementation of Ki67 assessment in practice is the lack of established guidelines on scoring Ki67 staining. In this study, we assessed the effect of nuclear hematoxylin counterstaining on visual perception of Ki67 positivity.

Methods

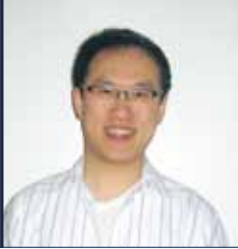
A single 1 mm core tissue microarray was constructed from 55 primary breast carcinoma samples. Serial sections from this TMA (N=14) were stained for Ki67 in 8 pathology labs in British Columbia. The slides were then digitized using the BLISS slide scanner and visually scored by a pathologist; percent of positive invasive breast cancer nuclei was recorded for each core. The coverslips were then removed from the slides, the hematoxylin was washed out with alcohol, and the slides were re-counterstained with hematoxylin simultaneously in one lab using a protocol optimizing contrast between nuclei and background. Restained slides were digitized scored by the same pathologist. Image analysis using ImageJ was performed on scanned slides to assess numbers of both positive and negative nuclei.

Results

Based on visual assessment of the original counterstained TMA slides, 6 of the 14 original lab counterstaining protocols yielded good contrast between nuclei and background, whereas it was difficult to distinguish the negative nuclei from background in the other 8 protocols (totalling 440 tissue cores). The image analysis program could not detect all negative nuclei in these latter cases, reflecting the weak nuclear counterstain. Comparison of visual scoring results before and after re-counterstaining showed a significant difference in the estimated Ki67 index; more negative nuclei were evident resulting in lower observed indexes after re-staining (mean $30.35 \pm 1.018\%$ before re-counterstaining and $27.98 \pm 0.985\%$ after re-counterstaining, $p = 2.52E-20$). When scores were dichotomized at a pre-established 10% cut-off point, the interpretation of 39 out of 770 cores changed from Ki67 high to Ki67 low after re-counterstaining, and 5 cores showed the reverse pattern. Image analysis of slides before and after re-counterstaining showed no changes in the positive nuclei count, proving that the process of counterstaining had not affected the DAB staining.

Conclusion

Weak hematoxylin counterstaining makes positively stained nuclei readily detectable, but leads to underestimation of the number of negative nuclei, resulting in increased estimates of the Ki67 index that could potentially result in overtreatment. Our data suggest that hematoxylin counterstaining is critically important in the assessment of nuclear immunostaining rates, and indicate the need to validate this aspect of immunostaining, possibly utilizing image analysis.



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EVALUATION OF CIRCULATING TUMOR CELLS AS PROGNOSTIC MARKERS IN PATIENTS WITH METASTATIC RENAL CELL CARCINOMA (mRCC) RECEIVING SYSTEMIC THERAPY

Background/Objectives

Recently, the enumeration of circulating tumor cells (CTC) in peripheral blood has been evaluated as a prognostic marker for various cancers including breast, prostate and colorectal. The presence of >4-5 CTC in 7.5 ml of peripheral blood before and/or after initiation of a new line of therapy is one of the strongest prognostic indicators of overall survival in these cancers. The Veridex CellSearch system is the only FDA approved commercially available assay. It is highly reproducible, specific and sensitive. As an adjunct to current standard testing methods, this assay has been used clinically to provide a more complete picture of patient prognosis. Our goal is to evaluate the potential utility of enumerating CTC in patients with mRCC.

Methods

Initially, patients in Canada with mRCC enrolled onto a phase III trial of second-line therapy of temsirolimus vs. sorafenib were evaluated for CTC. Blood was drawn at baseline, after cycle 2, and at time of treatment failure. Samples were processed on the CellTracks AutoPrep System[®] with the CellSearch Circulating Tumor Cell Kit[®]. Epithelial cells in the enriched sample were characterized and counted (CK+/DAPI+/CD45-) with the CellTracks Analyzer II[®].

Results

Samples from 12 patients were collected with a range of 0-38 CTC/7.5 ml of blood at baseline. Five of 12 patients (42%) had CTC counts of ≥ 5 . Nine of these patients had CTC counts performed after cycle 2: 4/9 (44%) had CTC counts ≥ 5 with 1 patient having a decrease in CTC counts from ≥ 5 to < 5 .

Conclusion

CTC are detectable in patients with mRCC and clinical follow-up of these patients continues. Enumeration of CTC on this study will be expanded to include all patients with mRCC receiving systemic therapy at the Vancouver Cancer Centre to further evaluate its use as a prognostic marker.

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David F. Schaeffer

Abstract # 9

ABSOLUTE INCREASE IN ENDOCRINE CELLS IN PEDIATRIC GASTRIC BIOPSIES FOLLOWING LONG-TERM PROTON PUMP INHIBITOR THERAPY

Background/Objectives

Long term gastric acid inhibition using proton pump inhibitor (PPI) therapy produces a marked increase in plasma gastrin, leading to expansion of the gastric oxyntic mucosa and hyperplasia of enterochromaffin-like (ECL) cells. Whether this leads to neuroendocrine neoplasms is unclear. Endocrine cell proliferative lesions have traditionally been classified as pseudohyperplasia, hyperplasia (diffuse, linear, micronodular, adenomatoid), dysplasia, and neoplasia (intramucosal or invasive carcinoids). This study was aimed at determining the ECL response to long term PPI therapy by measuring the absolute number of endocrine cells in the gastric mucosa pre- and peri- therapy.

Methods

20 pediatric patients (mean age: 8.2yr) received a pretreatment (baseline) gastric body biopsy and a 'treatment' biopsy following PPI therapy for at least 9 month (median: 26.95 months). Immunohistochemical staining for synpathophysin and chromogranin A was performed on all cases. Positive cells were counted in a blinded fashion in at least 5 crypts/biopsy and averaged. The Spearman rank-order correlation coefficient (r) was used to assess bivariate association.

Results

'Treatment' biopsies showed a 72% increase in Synapthophysin positive cells [pre: 649 (range/crypt 2-19); post: 1191(range/crypt 5-25)] and a 100% increase in Chromogranin A positive cells [pre: 563 (range/crypt 1-15); post: 1124 (range/crypt 3-20)]. Three cases showed discordant results by Synapthophysin staining (decrease in ECL cells during treatment), while no discordant cases were identified in the Chromogranin A stained cases. Synapthophysin expression correlated positively with Chromogranin A expression (r=0.07). Histomorphologically, all cases showed simple diffuse hyperplasia, without evidence of nodular changes.

Conclusion

The present study introduces a simple, accurate and reproducible method for counting ECL cells in the gastric mucosa and classifying them accordingly. We demonstrated an increase in endocrine cells in the majority of pediatric patients undergoing long-term PPI treatment. While the ECL increase was predominantly present as simple hyperplasia, the striking increase of ECL cells in this patient cohort, in conjunction with an increase in PPI treatment throughout our society, warrants careful monitoring of ECL hyperplasia and further investigation into the long-term outcomes.



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IDENTIFICATION AND CHARACTERIZATION OF A NOVEL TCF12-LIN28B FUSION IN SPORADIC WILMS' TUMOR

Background/Objectives

Wilms' tumor (WT) is the most common form of pediatric renal cancer; however, the genetics underlying the sporadic form of WT remain largely unknown. We recently characterized chromosome 6q21 breakpoints in two independent sporadic WT cases with t(6;15) translocations in an otherwise cytogenetically normal background. In both cases, we mapped the 6q21 breakpoints to an intergenic region between the HACE1 and LIN28B genes; the chromosome 15 breakpoint mapped to the TCF12 gene. LIN28B has been shown to have oncogenic properties through its ability to bind let-7 microRNAs (miRNAs) and reverse let-7 inhibition of multiple oncogenic pathways such as Ras and c-Myc. Furthermore, 6q21 rearrangements in the region of the LIN28B locus may be recurrent in WT based on multiple case reports in WT. We hypothesized that these t(6;15) translocations lead to the generation of a fusion transcript between TCF12 and LIN28B with oncogenic properties.

Methods

RT-PCR and sequencing was used to identify a putative TCF12-LIN28B fusion in WT samples. This fusion was cloned and expressed in NIH/3T3 and HEK293T cell lines for biochemical studies, including assays of let-7 levels, levels of let-7 targets, and transformation assays.

Results

Analysis of the index t(6;15) WT case by RT-PCR confirmed expression of a TCF12-LIN28B fusion transcript. Cloning and sequencing of the coding region of the full-length transcript showed fusion of exons 1-5 of TCF12 to exons 2-4 of LIN28B. RT-PCR for the fusion in the second t(6;15) case also demonstrated expression of the same fusion transcript, but was not detected in a small series of 10 other WT cases. When TCF12-LIN28B was overexpressed in NIH/3T3 cells, multiple let-7 miRNA family members were suppressed to a level similar to that seen with wild-type LIN28B overexpression. To establish the transformation ability of this fusion, we used the HEK-293T human embryonic kidney cell line to serve as a more relevant WT model. Again, akin to that seen with wild-type LIN28B, overexpression of TCF12-LIN28B in HEK-293T indeed promoted anchorage-independent growth in the soft agar colony assay. Furthermore, multiple let-7 targets are elevated in TCF12-LIN28B overexpressing cells compared to control cells at the protein level, including K-Ras and c-Myc. Paradoxically, the mitogenic response of both TCF12-LIN28B and LIN28B overexpressing cells to serum stimulation are reduced, based on levels of pERK and pMEK, and pAkt to a lesser extent, as readouts of Ras pathway signalling.

Conclusion

We identified a novel TCF12-LIN28B fusion gene in WT in the context of two cases bearing t(6;15) translocations. TCF12-LIN28B appears to possess oncogenic properties similar to wild-type LIN28B through their ability to modify let-7 miRNA levels and their downstream targets. Further studies are needed to determine the incidence of this fusion in WT and other cancers, and to elucidate the mechanisms by which it promotes WT tumorigenesis.

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Majid Zolein

Abstract # 11

A PERFORMANCE AUDIT OF ADRENAL VEIN SAMPLING IN BRITISH COLUMBIA: HOW ARE WE DOING?

Background/Objectives

Primary Aldosteronism (PA) is a treatable and often curable form of secondary hypertension affecting up to 10% of individuals who have a diagnosis of high blood pressure. Causes include, aldosterone producing adenoma (Conn's Syndrome), bilateral adrenal hyperplasia, glucocorticoid remediable hypertension, and other rare forms.

Because aldosteronomas are generally small, CT scan is unreliable to locate tumors. Therefore, once PA has been clinically proven by provocative studies, Adrenal Vein Sampling (AVS) is used to determine if there is lateralization of aldosterone production to one adrenal or the other. Success in AVS is technically hampered by the anatomy of the right adrenal vein which inserts directly into the IVC.

Our objective was to audit the utility and success of AVS procedures in BC and to evaluate commonly employed biochemical criteria for catheterization success. .

Methods

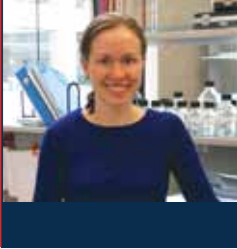
This was a retrospective audit of 90 cases of AVS which occurred from 2003-2010. Result of adrenal venous and peripheral venous cortisol and aldosterone were extracted from Vancouver Coastal laboratory information systems. All cases were systematically reviewed and calculations performed by standard approaches. Statistical analysis was performed using R (www.r-project.org).

Results

Numerous patients who did not have proven PA underwent the AVS procedure. Some patients had peripheral venous aldosterone results that actually excluded a diagnosis of PA. There was a statistically significant difference in the success rate of radiological operators with some having cannulation success rates of the right adrenal vein as low as 30%. Standard published biochemical catheterization criteria occasionally failed. ACTH stimulation during the procedure assisted in proving catheterization but obscured results on occasion. AVS helped localize adenomas that eluded CT and also helped identify some tumors as incidentaloma. CT prior to AVS assists in identifying anatomical variants.

Conclusion

AVS should be performed by a minimum number of operators. AVS utility in BC is inconsistent due to frequently failed cannulation of the right adrenal vein. All patients who undergo the procedure should have PA proven by a provocative test, with the exception of extreme presentations. ACTH stimulation during AVS is generally helpful provided its limitations are understood.



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MACROPHAGE RECRUITMENT AND TNF-ALPHA RELEASE: A PRO-INFLAMMATORY ROLE FOR HUMAN ISLET AMYLOID POLYPEPTIDE

Background/Objectives

Human islet amyloid polypeptide (hIAPP) is a 37-amino acid peptide co-secreted with insulin by pancreatic beta-cells. Pathological hIAPP aggregation occurs in type 2 diabetic and transplanted islets, both characterized by inflammation with macrophage infiltration. hIAPP fibrils share a common beta-sheet structure with other amyloids known to induce a potent inflammatory response via interaction with Toll-like receptors (TLRs) on innate immune cells. To determine whether hIAPP promotes islet inflammation, we evaluated the effects of hIAPP on monocyte recruitment to islets and on cytokine release by islets and macrophages.

Methods

Pro-inflammatory cytokines were measured in media from four-day cultures of hIAPP-expressing transgenic mouse islets and from hIAPP-treated bone marrow-derived macrophages. hIAPP-induced tumor necrosis factor-alpha (TNF-alpha) production was also evaluated in bone marrow-derived macrophages from mice deficient in TLR2, TLR4, or the TLR adaptor protein MyD88. THP-1 monocyte migration in response to supernatants from hIAPP-treated human islets was assessed by transwell assay. In vivo macrophage recruitment was evaluated by F4/80 staining of hIAPP islet grafts three months after transplantation into immunodeficient non-obese diabetic mice.

Results

Cultured islets expressing hIAPP produced significantly more macrophage chemoattractant protein-1 (MCP-1) than non-transgenic controls. Accordingly, conditioned media from hIAPP-treated human islets induced monocyte migration in vitro, and transgenic hIAPP islet grafts contained more F4/80-positive macrophages than wild-type grafts. Furthermore, hIAPP induced significant levels of TNF-alpha release from bone marrow-derived macrophages after 24 hours, but non-amyloidogenic rat IAPP did not. Interleukin (IL)-6, MCP-1, and IL-1beta were also upregulated. Cytokine induction was not associated with decreased macrophage viability but was blocked by Congo Red, an inhibitor of hIAPP fibrillogenesis. A significant reduction in TNF-alpha production was observed in MyD88- but not TLR2- or TLR4-deficient macrophages, and in cells treated with the IL-1 receptor antagonist Anakinra.

Conclusion

These data suggest that hIAPP-induced chemokine release by islets promotes macrophage recruitment. Direct interaction of macrophages with IAPP induces production of cytokines such as TNF-alpha and IL-1beta that promote beta-cell apoptosis. Furthermore, hIAPP fibril formation can activate MyD88 independently of TLR2 and TLR4, and MyD88-dependent IL-1 receptor signalling is at least partially required for TNF-alpha induction. Thus, inhibition of hIAPP aggregation in transplanted islets may represent a novel mechanism for suppressing the innate immune response.

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Ashish Marwaha

Abstract # 13

BIAS TOWARDS IL-17 SECRETING T CELLS IN CHILDREN WITH NEW ONSET TYPE I DIABETES

Background/Objectives

Type 1 diabetes (T1D) results from the autoimmune destruction of insulin-producing pancreatic beta cells. The role of regulatory T cells (Tregs) in suppressing autoimmunity has led to the hypothesis that their dysfunction may underlie T1D pathogenesis. Human studies assessing Treg dysfunction in T1D have used expression of the transcription factor FOXP3 to identify Tregs. However, distinct FOXP3-expressing Treg subsets have recently been identified based on the expression of the memory marker CD45RA and the staining intensity of CD25 (subunit of the interleukin-2 receptor). Notably, CD45RA- CD25^{int} FOXP3^{lo} cells lack suppressive function and instead secrete the pro-inflammatory cytokine interleukin (IL)-17. Since previous assessments of FOXP3⁺ Tregs in T1D did not assess changes in subsets of FOXP3⁺ cells, we re-evaluated whether the proportion of Tregs is altered in children with new-onset T1D.

Methods

Peripheral blood mononuclear cells were isolated from T1D subjects within six months of diagnosis and from age-matched controls. Flow cytometry, cell sorting and ELISA were used to delineate Treg subsets and assess IL-17 production.

Results

We report that T1D subjects have an increased proportion of CD45RA-CD25^{int} FOXP3^{lo} Treg cells, whereas other Treg subsets remain unchanged relative to controls. Sorting of CD4⁺FOXP3⁺ cells from T1D subjects indicates that the CD45RA- CD25^{int} FOXP3^{lo} subset secretes more IL-17 than other Treg subsets. Moreover, T1D subjects have a higher proportion of CD4⁺ and CD8⁺ T cells that secrete IL-17.

Conclusion

T1D subjects have an increased proportion of FOXP3⁺ and other T cell subsets that secrete IL-17. These data imply a potentially important role for IL-17 secretion in the pathogenesis of human autoimmune diabetes and suggest the possibility of therapeutic studies that target the IL-17 axis in T1D.



Rachel Wade

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ISHAK-KNODELL PATHOLOGY SCORE, MITOCHONDRIAL DNA CONTENT AND MITOCHONDRIAL GENE EXPRESSION IN LIVER FROM PATIENTS CO-INFECTED WITH HIV AND HEPATITIS C VIRUS

Background/Objectives

Hepatic mitochondrial toxicity is a concern for HIV/Hepatitis C Virus (HCV) co-infected patients and may be associated with altered mitochondrial DNA (mtDNA) content and mitochondrial gene expression (mt-mRNA) due to viral effects or associated therapies. We investigated the relationships between liver biopsy Ishak-Knodell (IK) pathology scores, liver mtDNA and mt-mRNA levels, in patients ON and OFF-highly active antiretroviral therapy (HAART).

Methods

In this observational cohort study, HIV/HCV co-infected patients (N=34 ON-HAART; N=18 OFF-HAART) underwent a double liver biopsy during pre-HCV therapy assessment. IK scores were recorded from patient charts. MtDNA/nDNA and mt-mRNA/beta-actin mRNA were quantified in the second biopsy sample by qPCR. Comparisons were performed using the Mann-Whitney test.

Results

There was high inter-individual variability in mtDNA and mt-mRNA, notably in the ON-HAART group (CV=92 and 103% respectively vs. 59 and 62% for OFF-HAART). The ON and OFF-HAART groups did not differ significantly in median[IQR] IK score (N=32, 7.5[4.8-9.0] vs. N=16, 7.0[4.0-8.3], p=0.84), mtDNA content (N=32, 368[315-545] vs. N=18, 399[312-623], p=0.81) or mt-mRNA (N=24, 28[19-36] vs. N=15, 27[21-30], p=0.92). No differences were seen between ON-HAART patients on protease inhibitors (PI) versus non-nucleoside reverse transcriptase inhibitors (NNRTI) with respect to IK scores (N=17, 8.0[4.0-9.0] vs. N=6, 6.0[4.3-8.5], p=0.74) or mtDNA (N=17, 391[305-538] vs. N=7, 320[242-394], p=0.19). However, mt-mRNA was higher in patients on PI compared to NNRTI (N=13, 34[26-39] vs. N=4, 17[15-20], p=0.015). Patients on dideoxynucleoside (D-drugs: d4T, ddI) versus other nucleoside reverse transcriptase inhibitors (NRTIs) did not differ in IK scores (N=6, 8.5[5.0-9.0] vs. N=24, 6.0[4.8-9.0], p=0.97), mtDNA (N=6, 357[302-502] vs. N=25, 370[320-625], p=0.68) or mt-mRNA (N=5, 35[21-39] vs. N=17, 27[20-34], p=0.54).

Conclusion

The lack of differences between the ON and OFF-HAART groups supports previous observations that HAART is not associated with increased hepatic mitochondrial toxicity amongst HIV/HCV co-infected patients. Although limited by small sample size, the results fail to associate drug classes (PI versus NNRTI; D-drug versus other NRTI) with toxicity. These findings may inform management of HIV/HCV co-infected patients.

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Amanda Vanden Hoek

IDENTIFICATION OF A NOVEL COAGULATION FACTOR X COMPOUND HETEROZYGOUS MUTATION ASSOCIATED WITH MODERATE BLEEDING

Background/Objectives

Factor X (FX) deficiency is a rare form of haemophilia (~1:2,000,000) characterized by a decrease in circulating coagulation FX antigen and/or activity levels which results in a variable bleeding diathesis. In heterozygous patients, bleeding may be absent or mild while homozygous and double heterozygous patients have phenotypes that are often associated with moderate or severe bleeding.

Patient History: In this study, a proposita now aged 70 with moderate bleeding diathesis is described. The patient was originally diagnosed as FX-deficient based on clinical measurements of coagulation factors and required 60 litres of plasma to arrest bleeding during prior minor surgery.

Methods

Plasma FX levels were assayed using both prothrombin time and activated partial thromboplastin time tests to evaluate the major initiating branches of coagulation, as well as western blots using FX-specific monoclonal antibodies. The entire FX gene (8 exons and flanking intronic sequences) was amplified using PCR and sequenced to identify mutations.

Results

Laboratory measurements of FX confirmed the clinical diagnosis, revealing that both the levels of FX antigen (30%) and activity (15%) were deficient. DNA sequence analysis identified two heterozygous mutations: a previously reported mutation which disrupts the splice site between exons 1 and 2 (IVS1 +1 G>A); and a novel mutation at nucleotide 28145 (C>T) which results in an Arg-386 to Cys (R386C) substitution in the serine protease domain of FX.

Conclusion

Here we have identified a novel FX mutation and provide the second report of a previously reported FX mutation. The IVS1 +1 G>A mutation was previously found to result in a 50% decrease in circulating FX antigen levels with Wang et al. (2005) hypothesizing that this mutation yields unstable mRNA in vivo and only the normal allele produced FX protein in the described heterozygous patient. In addition to reducing secretion, the novel R386C mutation appears to impair FX activity by approximately 50%, since ~30% antigen gave only ~15% activity in plasma. Future studies will introduce this mutation into recombinant FX to determine the precise details of inhibition by this mutation.



Farshid S. Garmaroudi

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DECONSTRUCTION OF THE COXSACKIEVIRUS B3-INDUCED SIGNALING NETWORK IN CARDIOMYOCYTES

Background/Objectives

Viral myocarditis caused by virus infection of the heart muscle is a common cause of heart damage and failure. Coxsackievirus B3 (CVB3) is one of the primary causative agents of viral myocarditis, particularly in children and young adults. To date, there is neither a vaccine to prevent viral infection nor a curative treatment beyond heart transplantation for viral myocarditis. Host cell-signaling molecules that support viral replication are potential drug targets for preventing CVB3 infection. Our laboratory has previously identified a collection of host cell-signaling molecules that individually contribute to CVB3 replication. Yet, it is unclear how this network of host factors is exploited by CVB3 during infection and virus propagation. Here, we propose a data-driven systems approach that deconstructs virus-induced host cell signaling into context-dependent molecule pairs that are mechanistically involved in CVB3 pathogenesis.

Methods

To study the CVB3-induced network, we generated a 3-dimensional dataset: i) variables: nine signaling molecules and two virus replication indicators; ii) timepoints: sham-infected cells [0 h post-infection (p.i.)], virus-receptor interaction (0.17 h p.i.), internalization (1 h p.i.), viral RNA synthesis (8 h p.i.), viral protein synthesis (16 h pi) and virion progeny release (24 h pi), and iii) 23 experimental conditions: (either one control, seven single signaling inhibitors or fifteen combinations of two signaling inhibitors). We used hierarchical clustering and graphical Gaussian modeling to identify time-dependent and global pairwise relationships between measured variables in the dataset.

Results

We predict from our analysis that: i) different mitogen activated protein kinases (MAPKs) play time-dependent roles in phosphorylating the activating transcription factor-2 (ATF2) and cAMP response element binding (CREB) transcription factors in the context of CVB3 infection; ii) inhibitor of NF-kappaB alpha protein (IkBa) plays a hub signaling role in assembling the overall network structure. In testing our prediction involving IB, we show that BAY11-7085 (a selective inhibitor of NF-kB downstream of IB) significantly reduces virus protein synthesis and viral progeny release.

Conclusion

Apparently, kinases are potentially targetable in viral myocarditis. To define a systems-level structure of signaling components may enable us to propose a more effective target (fewer side-effects) in treatment of the disease. This work shows how CVB3 infection is a host signaling network perturbation that can be simplified in a pairwise fashion to identify groups of molecules that are interrelated.

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Xin Ye

Abstract # 17

MICRORNA(MIR)-126 PROMOTES COXSACKIEVIRUS B3 REPLICATION BY ENHANCING ERK SIGNALING PATHWAY

Background/Objectives

Coxsackievirus B3 (CVB3) is the primary pathogen of viral myocarditis, a life threatening heart disease in infants and children. Unfortunately, there is no effective or specific treatment to this disease yet. More studies on the mechanism of CVB3 infection and pathogenesis of viral myocarditis are necessary to searching for new therapeutic targets. MicroRNAs(miRNAs) are small endogenous non-coding RNA molecules regulating the gene expression at the post-transcriptional level. To date, hundreds of miRNAs have been found to participate in various physiological and pathological processes including heart diseases such as myocarditis and cardiomyopathy. Mir-126 is one of the miRNAs enriched in the heart and plays an important role in vascular development and function. It regulates several fundamental signal pathways including the ERK (extracellular signal-related kinase) signal pathway. Here we investigated the effect of mir-126 on the CVB3 infection and pathogenesis of viral myocarditis.

Methods

Pre-mir-126 or negative control was transfected to HeLa cells for 48 hours. During the transfection, 24 h serum starvation or non-serum starvation was conducted to determine the effect of serum on signal activation. The live cell numbers and mir-126 level of the transfected cells were measured. The transfected cells were then infected with 10MOI CVB3 for 0-8h and the replication of CVB3 was determined by microscopy analysis of cell morphology, Western blotting to detect viral protein and viral plaque assay to measure viral titer. The ERK pathway signal proteins were also detected by Western blotting to elucidate the mechanism by which the mir-126 regulates CVB3 replication.

Results

In this study, we demonstrated the following: i). Compared with the control group, there was a 15% decrease in the total number of live cells in the pre-mir-126 transfected group. ii). The pre-mir-126 transfected cells exhibited significant cell elongation and membrane protrusions. iii). Increased levels of mir-126 led to more cell rounding and detaching as well as enhanced pro-Caspase-3 cleavage than the control cells during the CVB3 infection, indicating an increased cell death. The viral protein 1 (VP1) synthesis was also enhanced by the mir-126 transfection. The release of the viral particles was increased more than 100 times in the mir-126 group. iv). Infection of CVB3 downregulated the expression levels of SPRED1, a negative regulator of ERK signal pathway, correlating to the upregulation of phospho-ERK signal. Finally, v). The transfection of pre-mir-126 significantly enhanced the activation of ERK pathway by further reducing the expression levels of SPRED1 during the CVB3 infection in both serum-starvation and non-starvation conditions.

Conclusion

Our findings indicated that i) mir-126 may affect the cell proliferation and cell morphology; ii) Mir-126 plays an important role in facilitating the replication of CVB3 through the ERK signal.



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GRANZYME B CLEAVES PROTEOGLYCANS AND RELEASES SEQUESTERED TGF- β FROM EXTRACELLULAR MATRIX: IMPLICATIONS IN ATHEROSCLEROSIS AND PLAQUE INSTABILITY

Background/Objectives

Granzyme B (GrB) is a cytotoxic serine protease that contributes to chronic inflammation and disease through immune cell-mediated apoptosis and through the degradation of extracellular matrix (ECM). GrB resides in human atherosclerotic plaques and preliminary data in the apolipoproteinE-knockout model of atherosclerosis suggests that GrB localizes to the rupture-prone shoulder regions of advanced atherosclerotic plaques. We hypothesize that GrB degrades proteoglycans located in the cap region of atherosclerotic plaques and promotes plaque instability through the release of cytokines and promotion of inflammation.

Methods

For ECM cleavage assays, GrB, with and without the inhibitor 3,4-dichloroisocoumarin (DCI), was incubated for 4 and 24h, at room temperature, with decorin, biglycan or betaglycan and visualized by ponceau staining. Cleavage fragments were subjected to Edman degradation for cleavage site identification. As transforming growth factor- β (TGF- β) is sequestered by the aforementioned proteoglycans, GrB was incubated with TGF- β bound proteoglycans to determine if GrB cleavage results in the release of sequestered TGF- β . Cytokine release was assessed in supernatants using Western blotting. To determine if the TGF- β released by GrB was active, supernatants from the above release assay were incubated on human coronary artery smooth muscle cells (HCASMC) and Erk activation was examined by Western blotting.

Results

GrB cleaved biglycan, betaglycan and decorin, at both time points and cleavage was evident at GrB concentrations as low as 50 nM. This proteolysis was GrB-dependent, as cleavage was inhibited by the GrB inhibitor DCI and not by the inhibitor solvent control DMSO. Edman sequencing analysis determined GrB cleavage sites in decorin, biglycan and betaglycan with P1 residues of either aspartic acid or glutamic acid, consistent with the P1 specificity of the enzyme. In cytokine release assays, TGF- β was released from decorin, biglycan, and betaglycan in a GrB-dependent manner after 24h incubation. TGF- β was not released in the absence of GrB or when GrB was inhibited by DCI, thus there was no non-specific dissociation of TGF- β from decorin, biglycan or betaglycan. In addition, the TGF- β released by GrB remained active and induced Erk phosphorylation in HCASMC after 24h of incubation.

Conclusion

GrB cleaves decorin, biglycan, and betaglycan resulting in the release of sequestered active TGF- β . This may have implications in vivo, where cleavage of these structural proteins and a subsequent GrB-dependent increase of TGF- β bioavailability may promote plaque instability and atherosclerosis progression.

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Lisa Ang

Abstract # 19

SERPIN A3N REDUCES ABDOMINAL AORTIC ANEURYSM RUPTURE IN MICE BY INHIBITION OF EXTRACELLULAR GRANZYME B

Background/Objectives

Abdominal aortic aneurysm (AAA) is an age-related disease caused by progressive weakening of the vessel wall. Although AAA progression leading to rupture can be fatal, effective pharmacological interventions aimed at halting AAA progression at early stages of disease are not available. Previous work in our laboratory has demonstrated that knocking out the serine protease granzyme B (GZMB) reduces incidence and severity of AAA in mice. GZMB is well known for its role in eliminating target cells via apoptosis, but also accumulates extracellularly during inflammation and has been shown to cleave extracellular matrix (ECM) components such as fibronectin and fibrillin-1. The murine serine protease inhibitor, serpin A3N, has recently been identified as a novel and potent (IC₅₀<25 nM) extracellular GZMB inhibitor. We hypothesize that inhibiting extracellular GZMB will prevent the formation of AAA.

Methods

To induce aortic aneurysm, apoE^{-/-} mice were implanted with an osmotic minipump that released angiotensin II (1000 ng/min/kg) for 28 days. Animals were injected with serpin A3N (4-120 ug/kg) or saline control prior to pump implantation. Mice were euthanized and tissues harvested after 28 days. Survival, morphology, ECM composition and GZMB localization were evaluated. In vitro protease assays were performed to identify novel extracellular GZMB substrates that may contribute to AAA.

Results

Premature death from aortic rupture was observed in 50% of mice that received saline treatment, however, a significant dose-dependent reduction in the frequency of aortic rupture and death was observed in mice that received serpin treatment. Reduced GZMB positivity and degradation of medial fibrillin-1 and adventitial decorin was noted while collagen deposition was increased in the aortas of mice receiving serpin treatment. GZMB was also found to cleave decorin and biglycan *in vitro*.

Conclusion

GZMB contributes to the loss of vessel wall integrity in AAA through the cleavage of medial and adventitial ECM components. As treatment with serpin A3N promotes remodelling of the adventitia, reduces the rate of rupture and improves overall rate of survival, extracellular inhibition of GZMB warrants further investigation as a potential therapeutic strategy aimed at halting AAA progression.



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NOVEL SMALL MOLECULES THAT INHIBIT ANDROGEN RECEPTOR HAVE EFFECT ON TUMOR PROLIFERATION IN PROSTATE CANCER

Background/Objectives

Androgen receptor (AR) is a ligand-induced intracellular transcription factor that regulates genes involved in proliferation and survival of prostate cancer cells. In the nucleus, androgen-activated AR binds to androgen response elements (ARE) near the promoter or enhancer region of AR-regulated gene targets. With the recruitment of co-activators and assembly of transcriptional machinery, AR is able to initiate transcription. The objectives of this study were to identify novel small molecules that inhibit AR transcriptional activity; demonstrate specific inhibition of AR; and ultimately attenuate proliferation of human prostate cancer cells.

Methods

High throughput screening was applied to identify candidates that inhibit AR transcriptional activity, leading to the discovery of compound T1 and T2. Dose-response studies of T1 and T2 were performed by transient transfection of AR-specific luciferase reporter gene (PSA6.1-LUC) in LNCaP human prostate cancer cells, which contain functional AR and is androgen sensitive. Steroid receptor specificity was investigated in LNCaP by transfecting glucocorticoid receptor (GR) and progesterone receptor (PR) with their respective reporter genes. Effect on cellular proliferation was assessed by quantifying the incorporation of BrdU (thymidine analog) into the genomic DNA of aforementioned LNCaP, and PC3, which is an androgen-insensitive and AR-negative human prostate cancer cell line.

Results

Both T1 and T2 demonstrated significant inhibition on AR transcriptional activity in LNCaP at 1nM, comparable with that of bicalutamide, the first-generation antiandrogen used in clinic today to treat prostate cancer. Treatment of T1 and T2 did not cause cytotoxicity in LNCaP. Interestingly, T2 was shown to specifically inhibit AR but not PR, which has similar ligand-binding domain with AR; whereas T1 and bicalutamide both significantly decreased PR transcriptional activity. T1, T2, and bicalutamide all reduced cellular proliferation in LNCaP. As expected, proliferation in AR-negative PC3 cells were not affected by these compounds, indicating the inhibitory action of T1 and T2 is specific to the AR signaling pathway.

Conclusion

We discovered a novel small molecule T2 that inhibited AR transcriptional activity and reduced proliferation of cancer cells. Most interestingly, T2 demonstrated specific inhibition of AR but not PR, which shares the closest homology with AR within the nuclear receptor superfamily. The findings can be significant in the development of novel antiandrogens.

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Jennifer Kennett

Abstract # 21

CHARACTERIZATION OF GENOMIC ALTERATIONS IN A SUBSET OF RETINOBLASTOMA TUMORS USING HIGH RESOLUTION ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

Background/Objectives

Retinoblastoma is a rare childhood cancer of the retina. In Canada the survival rate is approximately 96%, this is largely due to early detection and treatment. However, worldwide only 50% of children survive their malignancy. Classically Retinoblastoma results from biallelic loss of the RB1 gene (M1 and M2) followed by accumulation of additional mutational events (M3-Mn). In 98% of tumors both RB1 mutations can be identified, however in the remaining 2% no mutation can be found (NMF). This subset of tumors will allow us to explore whether alternate mutational events can cause retinoblastoma. Understanding the role additional mutational events play in Retinoblastoma may give us greater insight into the biology behind the disease as well as possibly provide additional early screening techniques for the NMF group of patients.

Objectives

To comprehensively identify recurrent regions of copy number alteration (CNA) in Retinoblastomas with either RB1 mutational events found (RB1-/-) or not found (NMF) using whole genome tiling resolution array CGH analysis. We wish to identify both known and novel CNAs to provide insight into the underlying biology of these tumors.

Methods

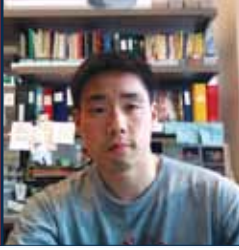
A sample set composed of DNA isolated from 24 NMF and 30 RB1-/- Retinoblastoma tumors was obtained from Dr. BL Gallie at Princess Margaret Hospital. Sample DNA and male genomic reference DNA were differentially labeled and co-hybridized to a submegabase resolution tiling-set (SMRT) array for CGH analysis. The SMRT array consists of approximately 27,000 overlapping bacterial artificial chromosome (BAC) clones spanning the entire human genome, enabling the assessment of genomic alterations at high resolution. Array data was assessed using custom SIGMA2 software and regions of CNA were defined and compiled for each sample.

Results

Whole genome array CGH profiles were generated for all 24 NMF and 30 RB1-/- Retinoblastoma tumor samples. By comparing profiles of the tumors with and without known mutational events we identified a higher frequency of MYCN amplification in the NMF Retinoblastoma tumors and an absence of many of the typical M3-Mn events. Previously reported regions of alteration such as those on 2p, 6p, 13q, and 16q containing MYCN, E2F3 and DEK, RB1, and CDH11, respectively, as well as novel alterations were identified in the Retinoblastoma tumors.

Conclusion

High resolution, genome wide copy number analysis enabled a level of comparison across tumors that has not been possible in previous profiling studies of Retinoblastoma. The identification of an increased frequency of MYCN amplification and a lack of the typical M3-MN alterations in the unique NMF Retinoblastoma tumor subset has raised some interesting questions.



Brian K. Chung

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NATURAL KILLER T CELLS KILL EPSTEIN-BARR VIRUS TRANSFORMED B CELLS FOLLOWING CD1D UPREGULATION BY RETINOIC ACID RECEPTOR-ALPHA

Background/Objectives

Natural killer T (NKT) cells are innate-like lymphocytes that recognize lipid antigens presented by highly conserved CD1d molecules. Individuals lacking NKT cells, such as patients with X-linked lymphoproliferative disease develop severe and often fatal lymphoproliferative disorders following Epstein-Barr virus (EBV) infection. Here, we show that EBV transformation of naïve human tonsillar B cells into lymphoblastoid cell lines (LCL) induces downregulation of surface CD1d but not MHC class I or MHC class II. Unlike naïve B cells, tonsillar LCL loaded with the exogenous NKT cell agonist alpha-galactosylceramide (alpha-GalCer) fail to activate NKT cells. To determine whether CD1d-dependent activation of NKT cells could be restored, LCL were treated with the synthetic retinoic acid receptor-alpha (RAR-alpha) agonist AM580 known to upregulate CD1d. Treatment with AM580 elevated transcriptional and surface expression of CD1d in LCL rendering them capable of stimulating IFN-gamma secretion and cytotoxicity by NKT cells, even in the absence of exogenous lipid agonists. When AM580-treated LCL were pulsed with alpha-GalCer, NKT cell effector functions were further enhanced. These results argue that EBV transformed B cells may evade NKT cell surveillance in vivo by downregulating CD1d expression and suggest that AM580 and alpha-GalCer may be used synergistically to augment NKT cell function.

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Cristina Tognon

Abstract # 23

CONTROL OF ONCOGENIC FUSION PROTEIN LEVELS BY THE INSULIN-LIKE GROWTH FACTOR RECEPTOR-I PATHWAY

Background/Objectives

Insulin-like growth factor-1 receptor (IGFIR) inhibitors are being tested in Phase I/II clinical trials for different types of cancers and have demonstrated significant response in a subpopulation of Ewings sarcoma patients harboring the EWS-Flt1 oncogene. The exact reason for this response is unknown however these agents have been shown to inhibit the Ras-Mek and PI3K-Akt pathways to decrease tumor cell growth and survival respectively. We have been studying the role of the IGFIR in cancers initiated by the expression of a dominant oncogene called ETV6-NTRK3 (EN). Our previous work identified an essential role for the IGFIR signaling axis in EN mediated tumorigenesis, particularly in the ability of these cells to survive in anchorage independent conditions. The objectives for this study were to determine whether a role for IGF-1 growth factor stimulation existed and whether blocking IGFIR activation using specific kinase inhibitors had any effect on tumor formation and downstream signaling using our EN tumor model systems.

Methods

We made use of four different cell lines that are transformed by EN, including murine NIH3T3 fibroblasts, IGFIR null (R-) and IGFIR+ (R+) murine embryo fibroblasts, murine breast epithelial cells (EpH4) and human breast epithelial cells (MCF10A). The effects of IGF-1 and insulin stimulation as well as IGFIR inhibition on: 1) signaling, 2) transformation, 3) migration, and 4) tumor formation was assessed using these cell line models.

Results

We show that EN transformed cells respond to IGF-1 stimulation by increasing EN phosphorylation. EN-mediated transformation and tumor formation are impaired in vitro and in vivo using IGFIR inhibitors and total fusion protein levels are affected by BMS-536924 a IGFIR/INSR kinase inhibitor. Inhibitor treatment does not decrease proliferation in monolayer cultures but induces cell death in an anoikis model. Using bimolecular fluorescence complementation (BIFC) assays in live cells we demonstrate that EN is ubiquitinated in response to BMS-536924 treatment and localized initially to small quick moving vesicles within the cytoplasm. MG-132 proteasome inhibitor treatment of these cells reveals an irregular aggresome to one side of the nucleus that is unrecoverable after photobleaching. Using different EN mutants we show that the effects observed after BMS-536924 treatment requires in part, the sterile alpha motif (SAM) domain or dimerization domain, found in the ETV6 portion of the fusion. These results raise the possibility that IGF-I/IGFIR activation may not only provide proliferative and survival signals to the tumor cells but could also control the level and/or activity of ETV6-containing fusion proteins. This mechanism could thereby allow for control of fusion protein expression and/or activity in response to IGF-I availability and influence growth accordingly.

Conclusion

IGFIR inhibition causes proteasomal mediated degradation of the ETV6-NTRK3 oncoprotein. Inhibiting IGFIR activity might therefore produce a larger than anticipated effect on cancer cell growth by blocking proliferation and survival initiated by IGFIR signaling and by subsequently downregulating ETV6-containing fusion proteins responsible for transformation.



POSTER PRESENTATIONS

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Naniye Cetinbas

Abstract # 24

HACE1 E3 LIGASE TUMOR SUPPRESSOR REGULATES PROTEASOME ACTIVITY

Background/Objectives

Hace1 is a tumor suppressor with HECT E3 ubiquitin ligase activity. E3 ubiquitin ligases attach ubiquitin (a 8,5 kDa protein) to a specific lysine residue on their target proteins in a tightly regulated manner. This post-translational modification is known as ubiquitination and it regulates many cellular events such as signaling, protein function and proteolysis. Our group previously showed that Hace1 gene is inactivated in human Wilms' tumors and Hace1^{-/-} mice develop spontaneous late onset tumors of diverse phenotypes. In addition, environmental stress, such as nutrient deprivation increases the tumor incidence. However, mechanism of Hace1's tumor suppressor activity and its E3 ligase targets are still unknown. This work aims to identify Hace1 E3 ligase targets under nutrient deprivation, which will help elucidate the mechanism of tumor suppression by Hace1.

Methods

We investigated Hace1 E3 ubiquitin ligase targets using a SILAC (stable isotope labeling with amino acids in cell culture)-based quantitative mass spectrometry under nutrient deprivation. HEK293 cells stably expressing GFP-tagged ubiquitin and Hace1 -or vector control- were grown in SILAC medium until the labeled amino acids were incorporated into entire proteome. At this point, all proteins in Hace1-expressing cells were labeled with heavy form of arginine/lysine, whereas the proteins in control cells were labeled with normal arginine/lysine. After nutrient deprivation treatment, cells were lysed, mixed 1:1, and ubiquitinated proteins were immuno-precipitated using GFP antibody. Proteins were identified and quantified by mass spectrometry analysis.

Results

We identified a number of proteins as potential Hace1 E3 ubiquitin ligase targets. Among these, we focused on the proteasome core particle (CP) subunit PSMA6 as we previously observed an interaction between Hace1 and proteasome CP. PSMA6 was ~ 3.5 times more enriched in Hace1 over-expressing cells. We also identified a lysine residue on PSMA6 that showed increased ubiquitination in Hace1-overexpressing cells. Proteasome is the main proteolytic machinery in the cell and its activity is upregulated in many tumors. We therefore hypothesized that Hace1 may regulate proteasome activity under nutrient deprivation. To test this hypothesis, we used fluorescence based in vitro and in vivo proteasome activity assays, and found that expression of Hace1 in HeLa cells significantly reduces proteasome activity under nutrient deprivation.

Conclusion

Our results indicate that Hace1 regulates proteasome activity by ubiquitinating the proteasome CP subunit PSMA6 at a specific lysine residue under nutrient deprivation. Approval of proteasome inhibitors such as Velcade for the treatment of relapsed multiple myeloma and mantle cell lymphoma validated the proteasome as a target for cancer therapy. Accordingly, our findings implicate the proteasome as a potential target in treatment of cancers, in which Hace1 is inactivated.



Crystal Leung

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HARVESTING VALUE FROM HUMAN TISSUES AT THE TIME OF CARDIAC TRANSPLANTATION: ONE STORY FROM THE CARDIOVASCULAR BIOBANK

Background/Objectives

The Cardiovascular (CV) Biobank was first established by Dr. McManus in 1982 at the University of Nebraska Medical Centre and was merged with the Pulmonary Biobank in the UBC Pulmonary Research Laboratory in 1993. The CV Biobank triages and archives a wide spectrum of human cardiovascular specimens, including native and prosthetic heart valves, blood vessels, medical devices, endomyocardial biopsies, and hearts removed at the time of transplantation or autopsy. This unique collection of biospecimens serves as a valuable resource for diagnostic, educational, and investigative purposes. Of materials in the Biobank, the heart specimens from cardiac transplantation procedures have been widely used in many collaborative research studies and educational endeavours. The CV Biobank has developed a standardized method for effectively archiving and biobanking heart specimens from cardiac transplantation, while preserving integrity to enable subsequent pathologic diagnosis.

Methods

Patients awaiting cardiac transplantation are consented for inclusion into the CV Biobank heart archive (PHC ethics H05-50208). Following removal of the heart during transplantation, the heart is picked up in heart collection (Hepes) buffer chilled on ice and brought back to the CV Biobank for archive. Gross images of each heart specimen are taken for documentation before the archiving process begins. The heart is given a Biobank ID - all tissues archived from this heart are identified by this number thus protecting patient privacy. Selected, standardized tissue samples, including myocardium, aortic and mitral valves, coronary arteries, and aortic and pulmonary arteries are archived, preserved with formalin fixation, OCT embedding, flash freezing, and RNA-later submersion. In addition to these preservation steps, aortic and mitral valve sections are prepared and processed for cell culture; these are stored in liquid nitrogen. Upon completion of the archival process, formalin fixed samples are submitted to the James Hogg Research Centre (JHRC) Histology Laboratory for processing and paraffin embedding. The OCT-embedded, flash frozen, and RNA-later specimens are organized, stored, and catalogued at -80°C. The remainder of the heart is placed in formalin and later examined for diagnostic purposes. .

Results and Conclusions

From 1996 to date, the CV Biobank has archived 218 explanted hearts from transplant recipients. This large collection of heart specimens has been integral to studies of protein constituents in cardiomyopathies, genetics of aortic valve stenosis, Biomarkers in Transplantation (BiT), and immunohistochemical character of failing hearts. The CV Biobank is a cornerstone of the James Hogg Research Centre as an important resource to foster elucidation of the mechanisms of disease and to accelerate health research with the intent of improving disease prevention, diagnosis, and treatment.

Crystal Leung, Amrit Samra, Lise Matzke, Mike Allard

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Crystal Leung

Abstract # 26

METHODOLOGICAL ISSUES RELATED TO PATHOLOGICAL EVALUATION OF CORONARY ARTERY STENTS

Background/Objectives

Intravascular placement of metallic stents is currently a common means of treatment for stenosed coronary arteries. Coronary stents are, however, associated with risk of in-stent restenosis and late-stent thrombosis which may lead to repeated target vessel revascularization or other life threatening conditions such as myocardial infarction or death. Pathological evaluation of stented coronary arteries is needed to document these complications and to characterize stent-tissue interactions in order to improve safety and efficacy of coronary stents. Thus, high quality tissue sections are required with excellent preservation of the morphology of stented artery, particularly at the stent-tissue interface.

Methods

Thirteen stented coronary artery segments from six cases of cardiac transplantation and seven routine autopsies, fixed in 10% neutral buffered formalin, were used. These specimens were dehydrated in an increasing graded series of isopropanol. In a vacuum chamber, the specimens were immersed in infiltration solution (benzoylperoxide with hydroxyethyl methacrylate) from the JB-4 Plus Embedding Kit® (Polysciences, Inc.). The specimens were then transferred into the embedding medium (infiltration solution with polyethylene glycol) and let polymerized under anaerobic conditions. After the medium has hardened, the specimens were cut into 5mm thick plastic blocks with a high-speed rotary saw. The plastic blocks were then affixed to the specimen holder using the embedding medium and sectioned with a JB-4 microtome (Sorvall®). The sections were subjected to hematoxylin and eosin staining and photomicroscopy was performed using the Nikon® CoolScope Digital Microscope.

Results

After successful processing, embedding, and staining, morphology of all 13 coronary artery segments, including the stent-tissue interface, was preserved and readily visible. To achieve adequate staining intensity, the sections of the stented arteries required prolonged hematoxylin staining. Some stents were displaced from their original implanted position during sectioning and staining. Attempts to use another plastic embedding medium (Technovit 9100 methyl methacrylate) have been unsuccessful so far. This is primarily related to failure of the block to adhere to the holder during sectioning.

Conclusions

Using the glycol methacrylate embedding technique, the morphology of the stent-tissue interface of stented arteries can be readily observed by microscopy. Failure to maintain the stent in its original position is an important limitation, possibly related to the relative softness of the medium. Refining the methodology may lead to a more efficient protocol and enable more specialized staining and immunohistochemistry as well as the use of harder plastic media such as methyl methacrylate.



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FATTY AND FIBROFATTY PHENOTYPES OF ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY IN SUDDEN DEATH AND HEART FAILURE: IMMUNOHISTOCHEMICAL ANALYSIS OF N-CADHERIN AND PLAKOGLOBIN

Background/Objectives

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetically determined heart muscle disease characterized by fibrofatty replacement of myocardium in the right ventricle (RV) and to lesser degree in the left ventricle. ARVC is commonly associated with sudden death and heart failure. Isolated infiltration of the RV by fat alone is also believed to be associated with sudden death. However, the ARVC phenotype versus that characterized by isolated fatty infiltration alone have an unclear separation. Several gene mutations encoding desmosomal proteins (including desmoplakin and plakoglobin) involved in linking adhesion molecules at the intercalated disk to the cytoskeleton - have been identified in approximately 40% of patients with ARVC [1]. While genetic testing for mutations in genes known to be associated with ARVC would aid in rendering a diagnosis, that approach is not practical in everyday pathology practice. One other possible strategy in better delineating phenotypic variation may be immunohistochemical (IHC) staining and quantitative evaluation of proteins related to genetic mutations underlying some ARVC phenotypes. This study examines the use of immunohistochemical staining for plakoglobin and n-cadherin on both fibrofatty and fatty phenotypes of ARVC in myocardium.

Purpose and Approach: In this study, we characterized heart case materials from forensic autopsy (7), cardiac transplantation (3) and endomyocardial biopsy (2) from patients with ARVC and fatty infiltration of the RV. Each case was referred to a cardiovascular pathologist at the Cardiovascular (CV) Biobank for assessment. Under approved ethics protocols, patient data were obtained from medical records or referring pathologists.

Methods

All case materials were archived between 1993 and 2009. All hearts were assessed for their macroscopic and microscopic features with confirmation by at least two observers. In this study, all autopsy heart specimens with available tissue for analysis were found to fit into one of two patterns: three cases demonstrated fibrofatty (2 male, 1 female, age = 24-36 years) replacement of the RV myocardium; four cases showed a pattern of predominantly fatty replacement (2 male, 2 female; age = 24-42 years). Quantitative computer-assisted morphometric analysis, done as part of a previous study, confirmed two pathological phenotypes, fibrofatty and fatty. Further, immunohistochemical (IHC) staining was performed on all heart cases for desmosomal proteins n-cadherin and plakoglobin.

Results

Fibrofatty replacement of the RV, characteristic of ARVC, and fatty infiltration of the RV alone are distinctive phenotypes in the setting of sudden cardiac death and heart failure. The results of this study indicate that reduced immunoreactive signal levels of plakoglobin at intercalated disks is a consistent feature in patients

with ARVC and is not seen in other forms of heart-muscle disease. Further, there is no significant difference in the immunoreactive signal levels of plakoglobin between fatty and fibrofatty phenotypes of ARVC.

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Elisabeth Anne Marie Matzke

Abstract # 28

CARDIAC AMYLOID IN THE SETTING OF TRANSCATHETER VALVE IMPLANTATION: A POSSIBLE CONTRIBUTOR OF POST-OPERATIVE DEATH?

Background/Objectives

Aging of the population and greater age-related aortic valve disease has led to an increasing number of elderly patients who are referred for heart valve surgery. Many of these individuals are not suitable for open heart valve replacement surgery and, instead, undergo transcatheter aortic valve replacement (tAVR). As many of these patients are beyond their seventh decade of life, certain conditions become increasingly common. One such condition is amyloidosis. Amyloidosis is characterized by the deposition of insoluble fibrillar protein aggregates in the heart and other tissue and is classified according to the protein precursor as immunocyte dyscrasia-related, hereditary, isolated atrial, reactive, and senile systemic amyloidosis (SSA). The SSA type is reportedly present in approximately one-quarter of autopsy cases in those over 80 to 85 years and, as such, is predicted to be a common finding in individuals undergoing tAVR that may contribute to post-operative mortality. In this report, we describe a series of autopsy heart cases from patients that underwent transcatheter valve implantation.

Methods

In this study, we characterized autopsy heart case materials from seventeen (17) patients that underwent transcatheter valve implantation at St. Paul's Hospital between 2005 and 2010. Each case was accessioned in the James Hogg Research Centre Cardiovascular (CV) Biobank at St. Paul's Hospital/University of British Columbia by the same cardiovascular pathologist. During pathologic evaluation, heart cases were routinely photographed and selectively radiographed. All hearts were assessed for their macroscopic and microscopic features in a standard manner, including detailed assessment of the stented prosthetic valve. Formalin fixed and paraffin embedded sections of myocardium were stained with hematoxylin and eosin and other special stains including congo red and sulfated alcian blue for amyloid. If appropriate, immunohistochemistry was performed with commercially available monoclonal antibodies directed against lambda (λ) and kappa (κ) light chains as well as against amyloid A protein.

Results

Over this time period, the 17 cases had a sex distribution of 10 males and 7 females with an overall mean age of 78.3 years (range: 57 to 99 years). The time from tAVR to death ranges from <1 day (perioperative death) to 2.5 years. Heart weights ranged from 358-921 grams. Key pathological observations included coronary atherosclerosis; myocardial infarction and ischemic injury; fibrosis; myocardial hypertrophy, injury to the anterior leaflet of the mitral valve, possible occlusion of coronary ostia by native aortic valve cusp, and localized injury of the atrioventricular conduction system. Of the 17 autopsy cases, 5 cases (2M; 3F, = 80.8 years) were found to have notable amyloid present. None of the amyloid deposits stained positively for light chains or amyloid A protein.

Conclusion

Amyloidosis with cardiac involvement, especially the SSA type, has been recognized in autopsy collections of the elderly. In this study, 29.4% of elderly patients who died following tAVR had notable cardiac amyloidosis and may have been a contributing factor to their post-operative mortality. The significance of amyloidosis to post-operative death is not fully appreciated and, given the global trend in aging and thus advanced age at time of surgical intervention, this possibility deserves increased consideration prior to surgical or transcatheter interventions.



Varun Saran

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MODULATION OF CARDIAC METABOLISM BY BETA-BLOCKERS DURING DIABETES: A ROLE IN APOPTOSIS SIGNALING

Background/Objectives

One cardiovascular complication associated with diabetes mellitus (DM) is a cardiomyopathy that can occur in the absence of valvular or ischemic heart disease and in normotensive patients with excellent glycemic control. Diabetic cardiomyopathy (DC) begins with asymptomatic diastolic dysfunction and can progress to heart failure if not managed. The cardiomyopathy observed may be due to a loss of contractile tissue as a result of apoptosis. Apoptosis may be caused by oxidative stress associated with metabolic and signaling modifications that precedes the development of dysfunction.

Chronic treatment with beta-adrenergic receptor antagonists (beta-blockers) improve function. Metoprolol and carvedilol are clinically important beta-blockers that have also been shown to favorably modulate metabolism; in addition, carvedilol, has been shown to have biologically relevant antioxidant properties. Preliminary in vivo data indicates that metoprolol can reduce apoptosis in the diabetic heart and improve cardiac output. Currently, there is no specific treatment for DC. Beta-blockers may serve as a novel treatment option

Methods

We employed a streptozotocin (STZ) induced rat model of type 1 DM. Animals received a one-time dose of 60 mg/kg body weight and the presence of DM was confirmed by measurement of blood glucose levels 3 days post injection. Two weeks post STZ, osmotic pumps containing β -blockers were installed. Metoprolol and carvedilol were delivered at a rate of 15 and 10 mg/kg/day, respectively. In order to assess the significance of carvedilol's antioxidant abilities, some metoprolol treatments were supplemented with vitamin C, delivered at 1000 mg/kg/day. A six and eight week time point were studied to assess changes during the asymptomatic and symptomatic phases of cardiomyopathy.

Results

Analysis of plasma indicates successful induction of DM, with an approximate 2.5-fold increase in blood glucose levels in STZ treated rats. Diabetic animals show increased fatty acid oxidation, that was normalized by beta-blockers and vitamin C and a significant reduction in rates of glucose oxidation, partially normalized only by both vitamin C with beta-blockers. However, animals that were diabetic for six weeks showed no DC as assessed by echocardiography and working heart perfusion.

Conclusion

Analysis of effects of beta-blockers, as well as assessment of protein expression, apoptosis and oxidative stress remains. The eight week time point is in progress.

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Anna Meredith

Abstract # 30

GENE AND PROTEIN EXPRESSION ALTERATIONS IN THE MYOCARDIUM FOLLOWING MECHANICAL CIRCULATORY ASSIST IN HEART FAILURE PATIENTS

Background/Objectives

The number of patients suffering from end-stage heart failure (HF) continues to mount alongside an aging demographic. Management of the failing heart involves medical therapy, mechanical circulatory assist (MCA) devices and ultimately heart transplantation. Unloading of the heart by MCA may induce reverse remodeling and normalize cardiac parameters (chamber geometry, heart size, ventricular volume, ejection fraction, fetal gene expression). We undertook the identification of markers within the heart correlating with patient response to MCA, and of changes in gene and protein expression in the myocardium following MCA.

Methods

Myocardial tissue cores removed during left ventricular assist device (LVAD) implantation, and explanted hearts obtained at the time of cardiac transplantation are archived for flash frozen, paraffin-fixed formalin embedded (PPFE), RNAlater and OCT preserved samples. Serial sections from PPFE samples of left ventricular (LV) apical tissue cores were prepared with standard histology stains (H&E, Movat[apos]s pentachrome and picosirius red) for quantification of fibrosis and degree of myocardial remodeling. Immunohistochemical staining of markers of myocardial dysfunction and remodeling (brain natriuretic peptide (BNP), galectin-3, versican, matrix metalloproteinases (MMP)-2 and -9, tissue inhibitor of metalloproteinases (TIMP)-1) were performed. RNA extracted from LV apical tissue cores was analyzed on Affymetrix GeneChip Human Genome U133 Plus 2.0 Arrays. Differential staining, gene expression and degree of remodeling were quantitated and correlated with patient response (non-responders: death, cardiac transplantation; responders: recovery with explantation of device). Comparison of gene and protein signatures from LV core samples at time of MCA initiation with myocardial tissue samples from the LV in matched explanted hearts was also performed.

Results and Conclusions

Increased galectin-3 and versican staining was seen in patients who did not respond to MCA, suggesting that activation of fibrosing pathways may impair the ability of the myocardium to recover despite chronic unloading. Differential gene expression of MMP-2 and -9 was observed in patients who subsequently required cardiac transplantation following MCA. Differential gene and protein expression was also seen in explanted hearts following MCA when compared to initial LV cores removed during MCA device implantation.



Verreault Maite

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LIPID-BASED FORMULATION OF IRINOTECAN (IRINOPHORE C), VINCRISTINE AND DOXORUBICIN TARGET TUMOR VASCULATURE IN GLIOBLASTOMA MULTIFORME)

Background/Objectives

Chemotherapy for the treatment of glioblastoma multiforme (GBM) patients is compromised due to the poorly perfused nature of glioma vasculature. Our laboratory reported that treating a subcutaneous colorectal tumor model with a liposomal formulation of irinotecan (Irinophore C) significantly changed vascular function and increased penetration and delivery of a second drug. The present study evaluates whether liposomal anticancer drugs have similar effects on the tumor vasculature of GBM models.

Methods

Commonly used anticancer drugs were selected via an in vitro screen based on a continuous low-dose cytotoxic drug exposure protocol previously described. Liposomal vincristine (2mg/kg), doxorubicin (®Caelyx; 15mg/kg) and irinotecan (Irinophore CTM; 25mg/kg) were injected i.v. in Rag2M mice bearing subcutaneous and orthotopic GBM tumors. Changes in tumor associated blood vessel morphology were quantified by immunohistochemistry: Microvessel density, angiogenesis, hypoxia, basal lamina integrity, pericyte coverage and blood vessel thickness. Overall functional aspects of the vasculature were assessed by injection of the marker Hoechst 33342 prior to termination.

Results

In addition to significant tumor size reduction (3.6-8.5 fold), normalization of the tumor vasculature was observed in both subcutaneous and orthotopic tumors. This effect was associated with an increase in Hoechst distribution in subcutaneous tumors (2-3 fold), and a partial restoration of the blood-brain barrier in orthotopic tumors, rendering it more impermeable to Hoechst and reducing its distribution in the tumor (5-11 fold).

Conclusion

These effects on the vasculature of GBM give rise to blood vessels that are morphologically more mature, and a network that is more evenly distributed in the tumor tissue. The normalization effects of Irinophore C on the delivery of a second drug (temozolomide) will be assessed in a near future.

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Jessica Kalra

Abstract # 32

SUPPRESSION OF HER2/NEU EXPRESSION THROUGH ILK INHIBITION IS REGULATED BY A PATHWAY INVOLVING TWIST AND YB-1

Background/Objectives

In a previous study it was found that the therapeutic effects of QLT0267, a small molecule inhibitor of Integrin Linked Kinase (ILK), were influenced by Her2/neu expression. This study was undertaken to assess the relationship between ILK and Her2/neu signalling.

Methods

To understand how inhibition or silencing of ILK influences Her2/neu expression, Her2/neu signaling in six Her2/neu positive breast cancer cell lines (LCC6Her2, MCF-7Her2, SKBR3, BT474, JIMT-1, and KPL-4) was evaluated using PCR, western blot analysis and immunofluorescence following treatment with QLT0267 to suppress ILK activity or siRNA used to silence ILK expression.

Results

Following treatment with QLT0267, these cell lines demonstrated suppression (32 to 87%) of total Her2/neu protein. Suppression of Her2/neu was also observed following siRNA mediated silencing of ILK expression. Time-course studies suggest that decreases in P-AKTser473, caused by ILK inhibition or silencing, were not temporally related to Her2/neu down-regulation. Attenuation of ILK activity or expression was, however, associated with decreases in YB-1 protein and transcript levels. YB-1 is a known transcriptional regulator of Her2/neu expression and here it is demonstrated that inhibition of ILK activity using QLT0267 decreased YB-1 promoter activity by 50.6%. ILK inhibition was associated with changes in YB-1 localization, as reflected by localization of cytoplasmic YB-1 into stress granules. ILK inhibition also suppressed TWIST (a regulator of YB-1 expression) protein expression. To confirm the role of ILK on YB-1 and TWIST, cells were engineered to over express ILK. This was associated with a 4-fold increase in the level of YB-1 in the nucleus, and a 2 and 1.5-fold increase in TWIST and Her2/neu protein levels, respectively.

Conclusion

Taken together, these data indicate that ILK regulates the expression of Her2/neu through TWIST and YB-1, lending support to the use of ILK inhibitors in the treatment of aggressive Her2/neu positive tumors.



Ramesh Saeedi

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STAVUDINE INDUCES INSULIN RESISTANCE IN CULTURED ADIPOCYTES THROUGH ALTERATION IN ADIPOKINE SECRETION AND ACTIVATION OF AMP-ACTIVATED PROTEIN KINASE

Background/Objectives

Stavudine, a nucleoside reverse transcriptase inhibitor, has been associated with an increased risk of insulin resistance (IR) and Type II diabetes mellitus (DMII) in patients infected with human immunodeficiency virus (HIV) mainly due to adipocyte mitochondrial toxicities and alterations in expression of adipocyte-derived adipokines. Adiponectin and retinol binding protein-4 (RBP-4) are two adipokines that are involved in controlling whole body insulin sensitivity. Adiponectin is a potent insulin sensitizing agent which its production seems to be dysregulated in conditions of insulin resistance. RBP4 is inversely correlated with expression of glucose transporter 4 (GLUT4) and has a major role in development of whole body insulin resistance (IR). AMP-activated protein kinase (AMPK), a regulatory kinase activated by metabolic stress, influences carbohydrate and lipid metabolism in response to the energetic status of the cells. Thus, we investigated the effect of stavudine on AMPK activation, glucose uptake and adipokine expression in 3T3-L1 pre-adipocytes in culture.

Methods

The 3T3-L1 cells, a permanent mouse pre-adipocyte cell line, were differentiated into an adipocyte-like phenotype in the presence or absence of stavudine (1-20 μ M) for 11 days. Adipocyte differentiation was evaluated at day 6 by oil red O staining of lipid droplets, and protein expressions of adiponectin and GLUT-4. On the 11th day of differentiation of 3T3-L1 adipocytes in the presence or absence of stavudine, uptake of [¹⁴C]-deoxy-D-glucose was measured following insulin stimulation. Release of adipokines by differentiated 3T3-L1 adipocytes was measured on the 11th day. Adiponectin and RBP-4 were measured from cell media by enzyme-linked immunosorbent assay (ELISA); additionally, AMPK activity from cellular lysate was measured on the 11th day.

Results

Upon differentiation, pre-adipocyte 3T3-L1 cells acquired adipocyte characteristics including up-regulation of adiponectin and GLUT-4, as well as cytoplasmic accumulation of triacylglycerol-rich droplets. Stavudine treatment of pre-adipocyte 3T3-L1 cells for 11 days during differentiation resulted in an increase in AMPK activity in a dose response manner. Stavudine treatment of pre-adipocyte 3T3-L1 cells also resulted in an increase in RBP-4 secretion and a decrease in adiponectin secretion in a dose response manner. Correspondingly, stavudine reduced insulin-stimulated glucose uptake in differentiated 3T3-L1 cells, also in a dose-dependent manner.

Conclusion

The results of this study suggest that stavudine activates AMPK in adipocytes and causes changes in adipokine secretion and glucose uptake indicative of insulin resistance. These findings provide a potential cellular mechanism that may contribute to stavudine-induced insulin resistance.

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Alisa Abozina

Abstract # 34

DEVELOPING A METHOD FOR ASSESSING THE EFFECT OF ANTI-RETROVIRAL HIV THERAPY ON PLACENTAL DEFENCE MECHANISMS

Background/Objectives

Pregnant HIV-infected women are given anti-retroviral therapy (ART), including nucleoside reverse transcriptase inhibitors (NRTIs), to prevent mother-to-child HIV transmission. However, the effects of these drugs on the placenta and the fetus are not fully understood. Several studies have shown that NRTIs cause mitochondrial toxicity, including mitochondrial DNA (mtDNA) depletion which can lead to mitochondrial dysfunction. Mitochondrial dysfunction is linked to the increased production of reactive oxygen species (ROS), which in turn can cause oxidative stress and thus induction of antioxidant defence mechanisms. An additional cellular defence mechanism involves the induction of a multi-drug resistance gene (MDR1), which pumps drugs out of the cell.

Objective: To develop a method that allows comparison of the induction of antioxidant and drug defence mechanisms in maternal and fetal placenta exposed to ART in-utero, with that of non-HIV infected control placenta.

Hypothesis: We hypothesize that the expression levels of antioxidant and drug-defence enzymes will be higher in placentae of HIV-infected and ART-exposed women than that in healthy controls.

Methods

The expression of catalase, peroxiredoxin-3, manganese superoxide dismutase, and MDR1 will be quantified with reference to GAPDH, a housekeeping gene, using primers that have been designed to amplify only the messenger RNA transcripts of the desired genes in real-time polymerase chain reaction (qPCR).

Results

Five primer pairs have been designed and tested using both PCR and qPCR, and have been shown to amplify only the genes of interest. The analysis of placental samples with the use of these primers in qPCR is ongoing.



Hayley Spencer

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LIVER ULTRASTRUCTURAL DAMAGE IN HEPATITIS C VIRUS AND HIV CO-INFECTED INDIVIDUALS

Background/Objectives

In the developed world, end-stage liver disease is the leading cause of death for patients infected with Human Immunodeficiency Virus (HIV). Among HIV-infected individuals, the Hepatitis C Virus (HCV) coinfection rate in Vancouver is estimated to be 50% (82% among intravenous drug users), in contrast to 33% in the developed world. Livers of coinfecting patients suffer repeated damage via many avenues, with HCV chronicity resulting in accelerated liver fibrosis and often hepatocellular carcinoma. Life-long management of HIV infection by HAART (Highly-Active Anti-Retroviral Therapy) includes the use of nucleoside-analog reverse transcriptase inhibitors (NRTIs), some of which are known to exert toxic effects on the mitochondria and decrease mitochondrial DNA (mtDNA). Mitochondrial toxicity can also result from anti-viral combination therapy for HCV; ribavirin (an NRTI) is used in conjunction with pegylated interferon to treat HCV infection. Treatment duration and efficacy vary depending on HCV genotype and other factors. To investigate the direct effects of ribavirin and pegylated interferon combination therapy in individuals on and off-HAART, we will employ morphometric and qualitative ultrastructural analyses to characterize liver biopsy specimens obtained before and after anti-HCV combination therapy. To do so, hepatocyte transmission electron microscope (TEM) images and mtDNA content will be examined before and after treatment.

Methods

Two ultrasound-guided 10mm/18 gauge needle liver biopsies are obtained from fasting coinfecting patients under local anaesthetic before and after HCV combination therapy. The first liver biopsy is examined by a single, blinded pathologist and scored for fibrosis and inflammation. The second biopsy is divided into 4 aliquots for analysis of DNA, for TEM imaging, and for future analysis of RNA and protein. mtDNA content will be analysed by quantitative PCR and expressed as a ratio of mtDNA to nuclear DNA, with a correction for the presence of binucleate hepatocytes. The TEM quarter of the biopsy is cut into 1mm³ pieces, and immediately fixed in 2.5% glutaraldehyde, sodium cacodylate buffered solution. After washes in buffer, specimens are post-fixed with osmium and potassium ferrocyanide, dehydrated, infiltrated, and embedded in epoxy resin (Epon). Thick sections (500 nm) are cut and stained with Toluidine Blue O for reference. Thin sections (60 nm) are cut and mounted on Formvar coated grids before staining with 2% uranyl acetate and Reynold's lead citrate. Sections are cut on a Leica EM UC6 ultramicrotome, and viewed in a FEI Tecnai 12 TEM at 80kV. Images are acquired with a Gatan BioScan 792 camera and associated Gatan software. Image-Pro software will be employed for morphometry, with a blinded single examiner.

Results

With careful analysis of morphometric data, we expect to observe ultrastructural changes after anti-HCV combination therapy in hepatocytes such as hypertrophy, changes in mitochondrial volume fractions, and evidence of altered metabolism such as changes in glycogen accumulation and lipid volume fractions. We also anticipate the possibility of alterations in other organelles such as peroxisomes, lysosomes and the endoplasmic reticulum. At the tissue level, there is a possibility of damage manifested as a change in overall biopsy score as described by the pathologist, and a measurable decrease in the mtDNA content of the hepatocytes.

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Tuhina Imam

Abstract # 36

PERIPHERAL BLOOD TELOMERE LENGTH IN INFANTS AND THEIR HIV-INFECTED MOTHERS TREATED WITH ANTIRETROVIRAL THERAPY DURING PREGNANCY

Background/Objectives

Zidovudine (ZDV) is routinely used in antiretroviral therapy (ART) during HIV pregnancy, to prevent vertical transmission. ZDV inhibits telomerase, the enzyme responsible for telomere elongation. We hypothesized that blood average telomere length (ATL) would be shorter in ART-exposed mothers and their infants compared to those who were untreated.

Methods

This retrospective cohort spanning 1990-2000 included HIV-infected pregnant women who were untreated, or treated with mono-, dual- or triple-therapy during their pregnancy. Maternal and infant dried blood spots (DBS) were collected at the last visit before delivery and at 0-6 weeks, respectively. DBS relative ATL was measured by qPCR. ATL were compared between treated and untreated mothers and infants using ANCOVA. Covariates included: maternal age, gestational age, smoking and recreational drug use. In linear regression analyses, additional possible predictors considered included, ART pre-pregnancy, duration of ART in pregnancy, maternal CD4 and pVL (plasma viral load) at last pre-delivery visit (when available) and age of infant at time of sample (infant ATL only).

Results

Infant ATL measured at a median [IQR] 1 [1-4] days of age were longer than maternal ATL ($p < 0.0001$) for both groups. However, ATL were not correlated in mother/infant pairs. No statistically significant difference in ATL was seen between treated and untreated infants ($N=81$, mean \pm SD 7.77 ± 1.54 vs. $N=39$, 7.90 ± 1.76 , $p=0.68$) or mothers ($N=81$, 5.93 ± 1.36 vs. $N=39$, 6.27 ± 1.21 , $p=0.19$), nor between mono/dual/triple therapy. Among the HIV-infected ART-exposed subjects, illicit drug use (ever) was associated with shorter maternal ATL ($p=0.04$).

Conclusion

No significant differences in maternal or infant ATL by treatment or HIV status were identified. This would suggest that if exposure to HIV/HAART is a risk for telomere attrition, it is less important than illicit drug use, which in turn may reflect Hep B/C virus coinfection, a highly correlated variable for which data was incomplete.



Eszter Papp

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EXPRESSION OF A MULTIDRUG-RESISTANCE PROTEIN (P-GLYCOPROTEIN) GENE (MDR-1) IN THE PLACENTA OF HIV INFECTED AND HAART-EXPOSED WOMEN

Background/Objectives

In HIV-positive pregnancies, developing fetuses are routinely exposed to highly active anti-retroviral therapy (HAART), components of which are known to cause mitochondrial damage. Through oxidative stress, mitochondrial dysfunction has been linked with telomere shortening, a biomarker of aging. The placental expression of the multidrug-resistance gene MDR-1, which can be triggered by drugs and oxidative stress, can reduce fetal drug exposure. The relationship between MDR-1 expression, mitochondrial toxicity and telomere shortening in placentae from HIV-infected HAART-exposed (study) and HIV-uninfected (control) women were investigated.

Methods

Placenta (fetal and maternal side) and infant blood from study (N=31) and control (N=23) women were collected and frozen immediately. Placental mitochondrial (mt) DNA, mt-mRNA, and MDR-1-mRNA levels as well as average telomere length (ATL) from blood and placenta were quantified by qPCR. The influence of HAART, prescription and illicit drugs on MDR-1 expression was explored. Pearson correlation and Mann-Whitney test were used for statistical analyses.

Results

In the placenta, MDR-1-mRNA levels were not significantly different between study and controls, but the fetal and maternal side were highly correlated, in the study group only ($R^2=0.585, p<0.0001$). Among mitochondrial toxicity markers, longer newborn blood (median [IQR] 1.0 [1.0-6.3] days) ATL were associated with higher placenta MDR-1-mRNA (on both fetal ($R^2=0.21, p=0.021$) and maternal ($R^2=0.17, p=0.038$) side). Similarly, placental ATL was also positively correlated with MDR-1-mRNA on the fetal ($R^2=0.353, p<0.001$) but not maternal side ($R^2=0.017, p=0.51$). MtDNA and mt-mRNA levels showed weak correlations with placental MDR-1-mRNA on the maternal (N=28, $R^2=0.205, p=0.016$, and $R^2=0.229, p=0.012$ respectively) but not fetal side. No clear relationship was seen between placental MDR-1 expression and infant blood lactate levels, duration of HAART or use of prescription or illicit drugs in pregnancy.

Conclusion

Our results suggest that MDR-1 induction plays a protective role against fetal drug toxicity, particularly with respect to telomere shortening, possibly by preventing mitochondrial damage in the placentas.

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Melissa Glier

Abstract # 38

CARDIOLIPOTOXICITY AND HEPATIC DYSFUNCTION IS AUGMENTED IN CBS +/- MICE WITH DIET-INDUCED OBESITY

Background/Objectives

Ectopic lipid deposition is a feature of obesity and associated with inflammation and oxidative stress. Cystathionine-synthase (Cbs) plays a role in glutathione synthesis, and is metabolically related to the methyl donor, S-adenosylmethionine (AdoMet). We hypothesize that mice heterozygous for deletion of Cbs (+/-) will be more susceptible to obesity-related lipotoxicity.

Methods

We studied +/-, and C57BL/6J (+/+) mice fed a high fat (60% energy) diet (HF) or a control diet (C) from weaning for 12 weeks.

Results

Mice (+/- and +/+) fed the HF diet had higher body weight ($P < 0.001$), greater visceral adiposity ($P < 0.001$), and higher liver triglyceride levels ($P < 0.05$) than +/- and +/+ mice fed the C diet. Cbs+/- mice had lower AdoMet levels ($P < 0.05$) and higher S-adenosylhomocysteine (AdoHcy) levels ($P < 0.001$) in liver than Cbs+/+ mice. HF-fed Cbs+/- and Cbs+/+ mice had lower liver AdoHcy levels ($P < 0.01$) than C-fed mice. Cbs+/- HF mice had lower liver AdoMet levels ($P < 0.05$) than Cbs+/- C mice. Cbs+/- mice had higher triglyceride levels ($P < 0.01$), higher Acox1 and Ppara mRNA ($P < 0.05$), and lower Acox1 protein ($P < 0.05$) in heart than Cbs+/+ mice. HF-fed Cbs+/- and Cbs+/+ mice had higher heart triglyceride levels ($P < 0.05$) than C-fed Cbs+/- and Cbs+/+ mice. Lower Ppara mRNA ($P < 0.01$) was found in heart from Cbs+/- HF mice than Cbs+/- C mice. .

Conclusion

These findings suggest that the Cbs+/- mice with diet-induced obesity are more susceptible to cardiac and hepatic lipotoxicity.



Serena Siow

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PROMOTING PATHOLOGY AND LABORATORY MEDICINE TO MEDICAL STUDENTS: THE UBC PATHOLOGY AND LABORATORY MEDICINE STUDENT INTEREST GROUP (PaLM-SIG)

Background/Objectives

Pathology is internationally recognized as an unpopular career choice among medical students. In Canada, only 1-3% of graduating medical students select pathological specialties for residency. This is despite a dire shortage of pathologists, which is projected to lead to declining quality of patient care. We seek to identify barriers to recruiting pathologists by exploring perceptions about pathology amongst medical students. We aim to search for existing initiatives which promote pathology. We also present an initiative that might help increase interest in pathology and laboratory medicine as a career choice.

Methods

Literature review and web search for original research, policy papers, and commentaries.

Results

We identified consistent themes with respect to perceptions about pathology that exist among medical students. These include: insufficient contact with pathologists; delayed exposure to pathology (after residency choice selection); misconceptions about how pathology is practiced; and limited knowledge about the relationship of pathology to other medical specialties. Current initiatives promoting pathology mostly involve curriculum changes; this approach does not address the barriers identified above. An alternative approach to increase recruitment, used by other specialties, is to support student-led interest groups. A limited number of student-driven initiatives in pathology do exist, mostly in the United States. Of the thirteen English-speaking medical schools in Canada, only one student interest group in pathology currently exists.

Conclusion

We establish the UBC Pathology and Laboratory Medicine Student Interest Group (PaLM-SIG) to help address the barriers identified. The goals of this new student-driven initiative are:

- 1) To expose medical students to pathology as a specialty;
- 2) To promote an appreciation for the importance of pathologists in patient care; and
- 3) To provide resources on shadowing, elective, and research opportunities.

We hope that our activities will encourage medical students to consider pathology and laboratory medicine as a career - or at least, to start loving the food analogies!

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Ahmad Al-Sarraf

Abstract # 40

CHARACTERIZATION OF PATIENTS WITH VERY HIGH PLASMA HDL-CHOLESTEROL, WITH AND WITHOUT VASCULAR PATHOLOGY

Background/Objectives

While epidemiological studies suggest that higher HDL-cholesterol (HDL-C) levels are antiatherogenic, very high levels of HDL-C may not be atheroprotective. There may be a tendency to underestimate cardiovascular risk in patients with very high HDL-C levels. We carried out a retrospective study of patients with HDL-C levels > 90th percentile to assess their cardiovascular status/risk.

Objective: To phenotype patients with very high plasma levels of HDL-C.

Methods

Methods and univariate statistics, the cohort had a lower incidence of smoking and physical inactivity, higher moderate alcohol intake, infrequent obesity and a healthy diet. There was a surprisingly low incidence of Type 2 Diabetes Mellitus (only two of 113 patients). A significant percentage of the patients had either evidence of cardiovascular disorder or high Framingham risk score (FRS). 15 patients had a personal cardiovascular history: 6 had suffered MI, 1 had positive stress test and 8 had carotid plaques. In addition, 16 had a FRS of greater than 20%.

Conclusion

Very high plasma levels of HDL-C in our cohort were associated with a healthy lifestyle and low incidence of diabetes. However, there was a significant number of individuals with preclinical or clinical vascular disease.



Shevaun E. Hughes

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DEREGULATION OF ONCOGENE AND TUMOR SUPPRESSOR EXPRESSION BY METHYLATION IN EARLY STAGE ORAL CANCER

Background/Objectives

Oral cancer is one of the world's leading causes of cancer death, with late stage disease diagnoses and high rates of recurrence accounting for poor survival rates. Like many solid tumors, oral cancer develops through a series of histopathological stages from hyperplasia to dysplasia, to carcinoma in situ (CIS), and finally to invasive disease. Understanding the initiating genomic alterations leading to gene dysregulation, and subsequent disease development, will lead to earlier and more directed treatment therapies. DNA methylation is a mechanism that can control gene expression and is known to be altered in several cancer types. Aberrant methylation has also been observed in premalignant lesions (such as p16 in oral dysplasia). Our goal is to identify genes that exhibit aberrant methylation and subsequent expression changes in the earliest stages of oral cancer progression, as early intervention allows for the greatest possible impact on patient survival.

Methods

We have profiled multiple histologically different (hyperplasia, dysplasia, and CIS) biopsies from 5 oral cancer patients. Gene expression and methylation status across the genome was determined using the Agilent 4x44K and Illumina Infinium platforms respectively. Using strict thresholds we compiled a list of candidate genes that exhibited decreased expression with a corresponding increase in methylation, and vice versa.

Results

As expected we observed a greater number of genes with increased expression and decreased methylation, with this trend holding true when we integrate the data from the two platforms. Despite the overall abundance of global hypomethylation, we observed a 2 fold increase in both the hypomethylation/increased expression and the hypermethylation/decreased expression from hyperplasia/dysplasia to dysplasia/CIS. Focusing on the hypermethylation/decreased expression candidates we have identified several candidate genes that exhibit this trend across multiple samples.

Conclusion

We have shown that there are a number of genes dysregulated in the early stages of oral carcinogenesis due to aberrant methylation patterns, which may be important in driving progression. Elucidating these genes could result in diagnostic markers and targets for therapeutic intervention, resulting in improved patient care and overall survival.

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Rebecca Towle

Abstract # 42

MOLECULAR CHARACTERIZATION OF ANAPLASTIC THYROID CARCINOMAS

Background/Objectives

Anaplastic thyroid carcinoma (ATC) is a very rare and aggressive disease. Though it makes up only 1-2% of all thyroid cancers, it accounts for half the deaths associated with this disease. Conversely, well-differentiated thyroid carcinoma, such as papillary (PTC) and follicular (FC), morphologies of thyroid carcinoma are much less aggressive and easily treatable. Histopathological review often shows that ATC occurs concurrently with either papillary or follicular carcinoma cell types, suggesting that these lesions could be ATC precursors. Parallel analysis of carcinomas from the same individual is needed to decipher this relationship and to uncover the basis for ATC tumorigenesis. By examining molecular alterations in ATCs and their putative precursor lesions we will identify the series of genetic changes that give rise to this aggressive disease. Unraveling these molecular mechanisms will provide great insight into ATC development which will lead to the development of novel targeted therapies.

Methods

Review of the cancer registry at the British Columbia Cancer Agency for the years 1975-2008 has identified ATC cases where microdissection is possible. Pathology review of this cohort will identify instances of concurrent ATC and either papillary or follicular thyroid carcinoma. Different tumor components have been isolated by microdissection, with DNA isolated from collected cells. DNA alterations were identified by a whole genome tiling-set array comparative genomic hybridization platform that has previously been demonstrated to yield robust data from archived tumor specimens. An automated DNA breakpoint detection algorithm was used to define genomic alterations in each tumor genome. Where identical DNA alterations and chromosomal breakpoints are detected for tumors from the same patient, a shared clonal origin was inferred.

ATC-specific alterations have been identified by subtracting the alterations from the precursor lesion. These unique alterations are likely the product of ongoing evolution, meaning that genes spanned by these alterations are likely critical factors in ATC development.

Results

To date we have analyzed several pairs of tumours (ATC and precursor lesion). Our preliminary results suggest a clonal relationship between the two cell types. We have also identified several alterations specific to the ATC genome. Potential candidate genes responsible for the transformation from PTC to ATC may map in this regions.

Conclusion

Anaplastic thyroid carcinoma a devastating disease. Preliminary results indicate a clonal relationship between PTC/FC and subsequent ATC. Several candidate genes have been identified that are specific to ATC development. These candidate genes will serve as future therapeutic targets for this otherwise untreatable disease.



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IS THE THIRD AFB SMEAR NECESSARY TO REMOVE PATIENTS FROM RESPIRATORY ISOLATION

Background/Objectives

Recent studies have suggested that two negative AFB smears may be as effective as three when screening patients for respiratory isolation purposes for *M. tuberculosis* (MTB). However, current recommendations in Canada, the United States, and Europe still support a three-smear approach.

Methods

The microbiology database of a tertiary care hospital was searched for sputum, tracheal aspirates and bronchoscopy samples from 2003-2007 that had been sent for mycobacterial testing. The first patient specimen to become AFB smear positive was noted. As well, the time required to collect the third specimen in hospitalized patients who remained smear negative was used to estimate the savings in isolation costs associated with a two-smear approach.

Results

There were 8347 respiratory specimens from 5168 patients in the five-year period. Of these patients, 2.2% (116/5168) were AFB smear positive, of whom 55.2% (64/116) were culture positive for MTB. Overall 89% (57/64) of patients were identified as being AFB smear positive by the first smear, 7.8% (5/64) were identified by the second smear and 3.2% (2/64) were identified by the third or greater smear. Smear negative patients spent 710 isolation days awaiting collection of the third sample at a cost of approximately CA\$142,000 over five years.

Conclusion

A two-smear approach for discontinuation of respiratory isolation precautions is a safe and cost effective.

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Paul Hiebert

Abstract # 44

SKIN AGING AS AN INDICATOR OF CARDIOVASCULAR HEALTH IN APOLIPOPROTEIN E KNOCKOUT MICE

Background/Objectives

The skin can be a useful indicator of cardiovascular aging and disease. Several studies suggest that markers of skin aging such as advanced glycation end products as well as skin conditions such as psoriasis and early onset alopecia are associated with increased risk of cardiovascular events including heart attack and stroke. We and others have observed an inflammatory skin aging phenotype in apolipoprotein E (apoE) deficient mice, an established animal model of atherosclerosis, which increases in severity when mice are fed a high fat diet. The purpose of the present study is to characterize the effects of apoE deficiency as well as diet on skin aging and disease in order to better understand the link between skin and cardiovascular health.

Methods

Wild type C57BL/6 and apoE knockout (apoE-KO) mice were grown to 0, 5, 15 or 30 weeks on either a regular chow or high fat diet. H&E, picosirius red and luna stains were used to assess morphology and extracellular matrix changes in formalin fixed skin sections. Multi-photon microscopy was also used to examine collagen organization and morphology in ex vivo mouse skin samples. Immunostaining for the cytotoxic protease granzyme B was also performed.

Results

ApoE-KO mice exhibited signs of frailty, hair loss and increased morbidity compared to wild type controls. H&E staining demonstrated a reduction in skin thickness along with increased inflammation and evidence of xanthomatous lesions while picosirius red and luna staining revealed marked differences in the collagen and elastin content respectively in apoE-KO mice compared to wild type controls. Multi-photon microscopy of thick skin sections revealed a decrease in collagen density and organization in apoE-KO mice compared to controls. Considerable granzyme B expression was also observed in the skin of apoE-KO mice. These observations became increasingly evident with age and when mice were fed a high fat diet.

Conclusion

These results suggest that, similar to its effects on plaque formation and destabilization, a high fat diet accelerates aging and disease of the skin in apoE-KO mice and further supports the concept of assessing the skin as a marker of cardiovascular health.



Gulisa Turashvili

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DNA QUANTITY AND QUALITY FROM PARAFFIN BLOCKS: A COMPARISON OF FIXATION, PROCESSING AND NUCLEIC ACID EXTRACTION TECHNIQUES

Background/Objectives

DNA extracted from paraffin blocks can be used for mutation detection, copy number analysis and most recently whole genome or exome sequencing using Next Generation Sequencing. Although the capacity to extract high quality DNA from paraffin blocks is of more importance now than ever, there is little information available to guide laboratories in their selection of tissue fixation, processing and DNA extraction techniques.

Methods

Nine samples (three each of colon, liver and muscle) were subjected to the following fixation and processing conditions: 1. frozen; 2. neutral buffered formalin (NBF) fixation <24 hours; 3. NBF fixation 7 days; 4. molecular fixative (MF) <24 hours; 5. MF 7 days. NBF-fixed samples were processed by standard processing (Tissue-Tek VIP5 processor, Somagen, Canada), and MF-fixed samples by rapid processing (Tissue-Tek Xpress, Somagen). DNA was extracted using phenol-chloroform manual extraction, RecoverAll (Ambion, USA), Waxfree DNA (Trimgen, USA), and QIAamp DNA FFPE Tissue Kit (Qiagen, Canada). DNA quantity and quality were assessed using Nanodrop, and PCR was attempted for amplicons of five lengths (268-1300 bp).

Results

Manual extraction yielded the highest amount of DNA followed by Waxfree DNA, QIAamp and finally RecoverAll kit, independent of the type of tissue, fixation or processing. For all five amplicons used, there were no significant differences between MF-fixed and frozen tissues, and between manual and Waxfree DNA kits on PCR. High molecular weight (1300 bp) DNA was preserved in MF-fixed tissues stored for <24 hours or 7 days, with no difference among the four kits ($p>0.05$). For NBF-fixed tissues, all kits performed similarly except for RecoverAll kit and manual extraction which amplified 536-989 bp and 536 bp fragments, respectively, in fewer samples than other kits ($p<0.02$). Short DNA fragments (268 bp) were successfully amplified in all tissue types using all kits.

Conclusion

The molecular fixative regardless of the duration of fixation, and the rapid processing system used in this study were able to preserve large DNA fragments in paraffin blocks, making these techniques suitable for use in molecular assays, particularly those involving sequencing technologies.

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Vincent Montoya

Abstract # 46

MICROBIAL SEQUENCE CHARACTERIZATION OF OVARIAN CANCER TISSUE

Background/Objectives

Epithelial ovarian cancer stands as the deadliest of all gynaecological malignancies. Among the heterogeneous subtypes of ovarian cancer, high-grade serous ovarian carcinomas (SOC) are clinically the most important. The known risk factors associated with SOCs correlate with and include a history of upper genital tract infections collectively termed Pelvic Inflammatory Disease (PID).

Methods

We sought to identify microbial sequences in SOC samples using 16S rRNA RT-PCR, a pan-viral microarray, and whole transcriptome sequence analyses using next generation sequence data.

Results and Conclusions

Our preliminary data provide evidence that SOC tissue is not as sterile as previously thought since bacteria, including some that are involved in PID and uterine tract infections, were recovered.



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20(S)-PROTOPANAXATRIOL AFFECTS THE EXPRESSION OF CYTOCHROME P450 3A4

Background/Objectives

Although ginseng and its active ingredients, ginsenosides, are used extensively as complementary and alternative medicines, their effects on the metabolism of concurrently used drugs are yet to be determined. Prolonged use of an herb may lead to changes in the expression of drug metabolising enzymes leading to clinically significant herb drug interaction. Cytochrome P450 3A4 (CYP3A4) is one such enzymes that is responsible for the metabolism of more than 50% of orally taken xenobiotics. We hypothesize that dietary intake of ginsenoside metabolites, 20(S) protopanaxatriol (aPPT), cause a change in the bioavailability of concurrently used drugs by altering the gene expression and enzymatic activities of CYP3A4. Specifically, the effect of aPPT on CYP3A4 gene expression and enzymatic activities in combination with 1,25- dihydroxyvitamin D3 (VD) in LS174T cells will be determined.

Methods

LS174T cells was treated simultaneously with or without 0.1nM or 1nM of VD and different concentration (0, 0.001, 0.01, 0.1, 1, 10, 50, and 100 μ M) of aPPT. Cell viability was determined by MTT assay. CYP3A4 protein expression was determined by western blotting. Total ginsenosides, Rg1, aPPD, and aPPT were tested for their abilities to inhibit CYP3A4 enzymatic activities in vitro using dibenzylfluorescein as a probe.

Results and Conclusions

aPPT (up to 10 μ M) alone or in combination with VD is not cytotoxic to LS174T cells. aPPT induces CYP3A4 protein expression with 0.1nM VD in a concentration dependent manner in LS174T cells. However, aPPT inhibits recombinant CYP3A4 enzymatic activities with IC₅₀ of 2.35 μ M.

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Walter Martz

Abstract # 48

DEATH BY BORIC ACID - WAS SUREKILLER® A SURE KILLER?

Background/Objectives

A 45 year old male was found deceased. Initial toxicological analyses did not reveal any involvement of alcohol, medications or drugs. A bottle of an insecticide (Surekiller®) was found at the scene. As the primary toxicology did not indicate an intoxication, the analyses focussed on the major ingredient of this insecticide - boric acid. The possible contribution of an ingestion of an insecticide containing boric acid to the cause of death is discussed.

Methods

Primary toxicology encompassed Liquid Liquid Extraction, Liquid Chromatography Mass Spectrometry (LCMS) and Gas Chromatography Mass Spectrometry (GCMS) screens, High Performance Liquid Chromatography (HPLC) equipped with Diode Array Detector (DAD) and Gas Chromatography (GC) equipped with Flame Ionization Detector (FID) or Nitrogen-Phosphorous Detector (NPD) for alcohol, drugs and medications. Boron was determined using Inductively Coupled Plasma Mass Spectrometry (ICPMS).

Results

No drugs, alcohol or medications other than Zolpidem (0.39 mg/L femoral blood) were detected. Boron was found in another aliquot of blood at 19 mg/L, in urine at 520 mg/L and in gastric fluid at 740 mg/L.

Conclusion

While boric acid is not particularly recognized as highly toxic, there is indication in the literature that it may have contributed to the cause of death in this case.



Josephine Cheng

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COMBINATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FLUORESCENCE DETECTION AND MODERN MASS SPECTROMETRY FOR ANALYSIS OF PRIMARY AMINE DERIVATIVES IN BIOLOGICAL FLUIDS AND TISSUES

Background/Objectives

Primary amines, such as amino acids, are important nutrients involved in many cellular processes. Amino acid levels are good indicators for diagnosis of healthy state or early onset of disease and thus often analyzed in clinical settings as for example in neonatal screening programs. Traditional analysis of amino acids is often done employing ortho-phthalaldehyde (OPA) and compounds with free thiols, such as mercaptoethanol (ME) or mercaptopropionic acid (MPA), for derivatization of primary amines followed by high performance liquid chromatography (HPLC) and fluorescence detection. Limitations of this method become apparent when different derivatives of primary amines co-elute from the HPLC column or when the derivatives are weakly fluorescent and are not picked up by the fluorescence detector. Here we present a method that combines the traditional approach of HPLC fluorescence detection with electrospray ionisation time-of-flight mass spectrometry (ESI-TOF MS) that allows a rapid identification of all eluting compounds and will also aid in quantitative studies when stable-isotope labeled analogues are used as internal standard compounds.

Methods

Biofluid samples, such as urine or plasma, are de-proteinized by addition of sulfosalicylic acid (SSA) to an end concentration of 4% followed by centrifugation. Tissue samples (e.g. liver) are homogenized and also treated with SSA for protein precipitation. Aliquots of de-proteinized samples are then diluted if necessary and combined with the derivatization reagent solutions containing OPA, MPA, buffers and additives. 10 μ L of the final reaction mixture is then analyzed by a Waters Alliance 2695 Separation Module equipped with a Waters 474 fluorescence detector. The separation column used is a Zorbax Eclipse AAA, 3.5 micrometer particle size and 2.1 mm inner diameter (Agilent). The 10 mM ammonium acetate buffered gradient is set to a 0.5 mL flow rate per minute. After the detector the effluent is fed into an ESI source of a Waters QTOFMS and a total ion chromatogram is recorded.

Results

Traditional HPLC separation of amine derivatives typically employs 40 mM Alkali phosphate buffers, which would be incompatible with ESI MS. For this reason we employed 10 mM volatile ammonium acetate buffers in our gradient that now enables the combination with ESI MS. We established a database of primary amines found in biological samples and included derivative structures and observed ion masses that enable rapid identification of eluting compounds. We are now in the process of applying this technology to clinical samples, such as urine samples from children with Cystic Fibrosis, since a previous study has indicated glycine conjugation irregularities. Other applications include samples from ongoing collaborations such as rat liver tissues from different diet groups and cell culture samples to determine growth determining factors. In the latter case we already established taurine deficiency in cell cultures substituted with dialyzed serum compared to non-dialyzed serum and were able to identify a previously unknown contaminant in some samples.

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Katelyn Janzen

Abstract # 50

PROTEIN TARGETS REGULATED BY POLYUNSATURATED FATTY ACID MATERNAL DIET IN FETAL RAT BRAIN

Background/Objectives

Polyunsaturated fatty acids (PUFA) are important nutrients for brain growth and development. Omega-3 or n-3, and omega-6 or n-6 fatty acids cannot be synthesized in humans and need to be supplied with diet. The amount and type of PUFA consumed by the mother is passed on to the fetus through placental nutrient transfer; this is the time when the brain grows most rapidly, requiring adequate PUFA levels for healthy development. N-3 PUFA can be found in fatty fish, canola oil and flaxseed oil, whereas n-6 PUFA can be found in soybean, safflower and sunflower oil. The typical western diet contains much more n-6 PUFA compared to n-3 PUFA and since both groups compete for the same enzymes this can limit the benefits of n-3 PUFA, even if consumed in high amounts. It is known that PUFA regulate gene expression, but a comprehensive study on which genes are affected by either a high or low levels of dietary n-3 PUFA has not been done yet. Here we present a proteomics study where we identify differentially expressed proteins in fetal rat brain, as a result of a high or low level maternal n-3 PUFA diet.

Methods

From two weeks before and throughout gestation, maternal rats were started on a high or low n-3 PUFA diet, with the dietary fatty acids coming from canola oil (high n-3 PUFA) or safflower oil (low n-3 PUFA). Two days before delivery fetal rats were removed by C-section. Brain homogenates from the pups were prepared for two-dimensional polyacrylamide gel electrophoresis (2D PAGE). Gels were stained with Coomassie Blue and the scanned images compared between groups with PDQuest software (BioRad). Spots of differentially expressed proteins were excised from the gel. In-gel digestion with trypsin was performed, resulting peptide mixtures extracted and analyzed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI TOF MS). Peptide fingerprint maps were submitted to the MASCOT search engine (www.matrixscience.com) and proteins were either identified via their peptide fingerprints or peptide fragmentation spectra.

Results

To date we tentatively identified 13 proteins between the two groups. Five proteins were expressed to saturation and were identified for quality control purposes. Another two proteins showed equal expression levels and were also identified for control purposes. Five proteins were found to be downregulated in the high n-3 PUFA group and these were identified as 14-3-3 zeta/delta, Protein disulfide isomerase A3, Vimentin, heterogeneous nuclear ribonucleoprotein and a phosphatase 2A.

Conclusion

2D PAGE combined with mass spectrometric identification of separated protein spots is an effective tool in discovery of protein markers that are regulated by individual nutrients stemming from the maternal diet in nutritional studies. Further studies will look at the involved important metabolic pathways in early development, now that we have identified critical protein targets.



Gao Guangzheng

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SHORT ANTIMICROBIAL PEPTIDES IMMOBILIZED COPOLYMER BRUSHES ON TITANIUM SURFACE

Background/Objectives

Implant-associated infection is one of the most challenging postoperative complications, especially in the case of orthopedic implants. Thus the design of novel anti-infective implant surfaces would be highly advantageous with significant benefit to both patients and the health care system. A potential alternative to current antibiotics is antimicrobial peptides (AMPs) that are found in the immune system of almost all species of life.

In the present work, we investigated the immobilization of small AMPs on titanium surfaces using novel functional copolymer brushes. We show that the properties of the copolymer brushes significantly affect the peptide density on the brushes and their antimicrobial activity.

Methods

We synthesized of primary amine functionalized copolymer brushes on Ti surface using surface initiated atom transfer radical polymerization (SI-ATRP) technique and developed methods for the efficient conjugation of cysteine containing AMPs on copolymers brushes and their characterization. Antibacterial analyses were conducted by incubating Tet-213 immobilized Ti plates and corresponding control samples in a 24-well microtiter plate with lux strain culture. Luminescence emission was examined by a TECAN® Spectrafluor Plus spectrometer (TECAN U.S., Inc.).

Results

N-(3-Aminopropyl)methacrylamide hydrochloride (APMA) and N, N-Dimethyl acrylamide (DMA) copolymer brushes were synthesized on Ti surface by surface initiated ATRP in aqueous conditions at room temperature. Cysteine-containing AMP Tet-213 was conjugated to the copolymer brushes after modification of amine groups to maleimide groups. The antimicrobial properties of the selected peptide-coated samples were analyzed using *Pseudomonas aeruginosa* (*P. aeruginosa*) strain PA01 that expresses a luciferase gene. Our preliminary data show that the polymer brushes grafted Tet-213 retains antimicrobial activity. Bacterial growth inhibition could reach 80%.

Conclusion

We showed that the primary amine groups within the brushes were highly reactive to small molecules, and almost quantitative conversion of amine groups to maleimide groups was obtained. The peptide conjugation of copolymer brushes depended on molecular weight, graft density and composition. The graft density of the chains on the brushes showed significant influence on the peptide conjugation unlike other parameters. Many folds higher peptide density on the surface was achieved when the brush layer was used for conjugation. As a proof-of-concept, we showed that peptide conjugated copolymer brushes were antimicrobial.

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Abstract # 52

MACROMOLECULAR IRON CHELATORS: A NOVEL THERAPEUTIC AGENT FOR THE TREATMENT OF SECONDARY IRON OVERLOAD

Background/Objectives

Transfusion therapy has improved the quality of life and prolonged survival for many patients with inherited and acquired refractory anemias such as sickle-cell and thalassemia. However, the human body lacks a physiological mechanism to actively excrete excess iron; therefore, repeated blood transfusions lead to increased body iron burden or secondary iron overload. Excess iron catalyzes the formation of very injurious compounds, such as the hydroxyl radical, which is highly reactive, and attacks lipids, proteins, and DNA in major organs. Additionally, iron-mediated carcinogenesis is thought to occur through the generation of oxygen radicals. Iron overload is thus involved in the pathogenesis of many diseases. Desferrioxamine (DFO) is the most widely used iron chelating drug, but has significant disadvantages, including its high cost and continuous subcutaneous mode of administration for 8-12 hours per day, 5-7 days a week. Since molecular weight (MW) strongly influences the cell permeability, toxicity, and plasma half-life of drug molecules, we hypothesize that a macromolecular system with iron binding groups attached to a biocompatible polymer will reduce the systemic toxicity and increase plasma residence time of current iron chelators thereby increasing the therapeutic efficiency.

Methods

Our group has recently developed a novel blood compatible hyperbranched polyglycerol (HPG) based iron chelator system. We conjugated HPGs of defined MW to low MW DFO. HPGs were initially oxidized with sodium periodate (NaIO₄) to generate reactive aldehyde groups from 1,2 diol groups on the HPGs. Aldehyde groups were coupled with DFO via Schiff base reaction followed by reduction with sodium cyanoborohydride (NaCNBH₃). Remaining aldehyde groups were quenched using excess glycine or ethanolamine. The iron-binding efficiency as well as the ability of HPG-DFO conjugates to prevent iron mediated oxidation of proteins were determined by UV-Visible Spectroscopy. Blood compatibility of these conjugates was analyzed by coagulation assays including Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and Thromboelastography (TEG); platelet activation, and red cell aggregation.

Results

Characterization of novel HPG-DFO conjugates using UV-Visible Spectroscopy demonstrates that the iron binding properties of DFO remains unaffected after conjugation. Additionally, HPG-DFO was effective at preventing iron-mediated oxidation of our hemoglobin oxidation model. Blood compatibility analysis did not show any significant changes in blood coagulation, platelet activation, hemolysis, or aggregation *in vitro*.

Conclusion

The synthesis of a novel class of iron binding, HPG based chelator is described. The iron binding property of DFO is not hindered once conjugated to the HPG. Blood compatibility analyses indicate that the new polymer-based chelator is highly biocompatible. Currently we are continuing studies on cell toxicity and *in vivo* analysis of these molecules in mice. A successful outcome would benefit millions of people, especially iron overloaded sickle-cell and thalassemic patients.



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SYNTHESIS OF BIOMIMICKING GLYCOPOLYMER BRUSHES CONTAINING DIFFERENT CARBOHYDRATE RESIDUES AND THEIR NON-BIOFOULING PROPERTIES

Background/Objectives

The glycocalyx is a “carbohydrate-rich coat” on the external surface of the plasma membrane of cells, playing essential role in cell recognition, cell adhesion, protection and permeability barrier. Moreover, the glycocalyx also serves as a layer which prevents the undesirable nonspecific adhesion of other proteins and cells. Several approaches have been used to develop glycocalyx-mimetic surfaces to prevent non-specific adsorption either by physical adsorption or covalent coupling carbohydrate containing polymers onto the surface. However, those surfaces possess low carbohydrate density or being lack of functional carbohydrate residues. Thus, the development of surface with high carbohydrate density and different carbohydrate species will address the role of carbohydrates in preventing non-specific adsorption and provide a better non-biofouling surface.

Methods

Three glycopolymer brushes containing different carbohydrate units were prepared on silicon substrates by surface-initiated atom transfer radical polymerization (SI-ATRP). The formation of glycopolymer brushes were characterized by ellipsometry, ATR-FTIR, water contact angle analysis and X-ray photoelectron spectroscopy. The kinetics of polymerization in solution was thoroughly studied by GPC and NMR. The capability of prepared surfaces against adsorption from bovine serum albumin (BSA) and fibrinogen (Fb) solutions was investigated by using fluorescence microscope.

Results

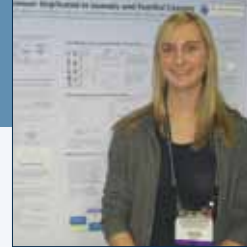
Three novel N-substituted acrylamide derivatives containing different carbohydrate units (mannose, galactose and glucose) were synthesized by the protection-deprotection method. Homo-glycopolymer brushes were prepared by SI-ATRP of above monomers. The utilization of CuCl/CuCl₂/Me6TREN as the catalyst, H₂O as the solvent gave better control of brush growth compared to other systems. The kinetic study showed that the initial rate of the polymerization in solution and growth of polymer brushes was very fast. The resulting three glycopolymer brushes showed super-low protein adsorption from bovine serum albumin (BSA) and fibrinogen (Fb) solution.

Conclusion

Our results indicate that the glycopolymer brushes show good performance in preventing non-specific protein adsorption. We anticipate that this new glycopolymer brushes may have applications in the development of novel non-biofouling surfaces for medical implants, tissue engineering matrixes, drug delivery systems, biosensors and diagnostic array.

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Catherine Enfield

Abstract # 54

A NOVEL LUNG TUMOR SUPPRESSOR IMPLICATED IN SOMATIC AND FAMILIAL CANCERS

Background/Objectives

Lung cancer (LC) is the most common cause of cancer death worldwide. Previous familial linkage studies have identified a tumor suppressor locus on 6q23-25. However, no single gene has yet been implicated within this 30 Mb region. Discovering the genetic and epigenetic events that affect LC risk and development will lead to better methods for risk assessment, early detection and treatment.

Methods

Genome-wide genes disrupted by two-hit inactivation were identified by combining gene dosage, DNA methylation, and gene expression assays for a group of lung adenocarcinomas (AC) and adjacent non-malignant tissues. Gene expression, DNA hypermethylation and/or copy number aberrations were validated in data from AC, squamous cell carcinoma (SqCC), and pre-malignant lesions by querying other cohorts using gene-specific and whole-genome approaches. The role of DNA methylation in gene silencing was assessed using inhibition of DNMT by 5'-azacytidine. The association of allelic variants with LC risk was investigated in 193 familial LC cases and 213 controls collected by the Genetic Epidemiology of Lung Cancer Consortium (GELCC) using a Cochrane-Armitage trend test. The association of gene expression with prognosis was performed on public data using a Mantel-Cox log test. Stable mRNA knock-downs were generated using lentiviral delivery of a gene-specific shRNA, and apoptotic cells were counted using Annexin5/propidium iodide staining.

Results

Integration of AC gene dosage, DNA methylation and mRNA expression showed EYA4 to be frequently affected by two-hits and significantly down-regulated. Quantitative PCR techniques confirmed that EYA4 was hypermethylated (46%) and down-regulated (72%), validating our microarray results. A direct link between EYA4 methylation and expression was verified by restoration of expression after 5'-azacytidine treatment in methylated cell lines. Congruent with EYA family member function, in vitro assays revealed that EYA4 knock-down cells displayed a decrease in the number of apoptotic cells - a hallmark of cancer. Further investigations led to the discovery of frequent EYA4 disruption in SqCC and pre-neoplastic tissue. The GELCC dataset was examined to assess EYA4 allelotype association with familial risk. Doing so revealed that numerous EYA4 variants are associated with increased risk. Finally, the association of EYA4 expression with survival was investigated along with other somatically altered genes at 6q23-25. Of these genes, low EYA4 expression was found to be the most significantly associated with poor prognosis.

Conclusion

EYA4 is a frequently disrupted gene that maps to a locus previously associated with cancer risk. It is implicated in somatic as well as familial cancers, and is likely a tumor suppressor gene with apoptotic functions. The direct association of EYA4 with risk and survival underscores its relevance on a clinical level.



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AMPLIFICATION STATUS OF GAS4I AND MDM2 IN LUNG ADENOCARCINOMAS

Background/Objectives

Lung cancer is the leading cause of cancer death worldwide. High mortality rates are attributed to inefficient therapeutic strategies and a lack of screening for early detection. Identification of genes involved in lung cancer development may yield new molecular targets for diagnosis and treatment. It is well documented that the lung cancer genome harbors genetic alterations. We hypothesize that novel oncogenes can be discovered through characterization of chromosomal regions frequently amplified in lung adenocarcinoma.

Methods

A panel of 169 matched lung adenocarcinoma tumors was studied. Genetic profiles describing copy number alterations and gene expression were generated by whole genome array comparative genomic hybridization and Affymetrix platforms respectively, and visualized using SIGMA2 software. Regions of DNA amplifications were identified using the GISTIC algorithm and integrated with expression data to determine if amplification is associated with overexpression in tumors relative to matched normal tissue. An MTT assay was used to investigate the effect of cell proliferation after shRNA-mediated knockdown in a cell line with DNA amplification. Survival analysis using multiple independent data sets was also performed to assess the clinical significance of gene amplification and overexpression.

Results

A preliminary genome wide screen revealed Glioma Amplified Sequence 41 (GAS41) at 12q13-15 as frequently amplified and overexpressed in lung cancer. GAS41 suppresses cell cycle checkpoint genes p21 and p14, causing indirect repression of the p53 tumor suppressor, promoting tumorigenesis. Amplification of this gene has been reported in the earliest stages of glioma and astrocytoma. GAS41 is amplified in 16.57% of the lung adenocarcinoma tumors (28 of 169) analyzed and consistently overexpressed. Knockdown of GAS41 slowed cell proliferation, consistent with its biological function. Additionally, patients expressing high levels of GAS41 mRNA showed markedly poorer outcomes than those with lower expression.

Conclusion

Our data highlights the tumorigenic potential of GAS41, suggesting that amplification of this gene may be a critical alteration in lung cancer. GAS41 amplification and subsequent activation may represent a novel mechanism driving lung tumorigenesis. The association of GAS41 expression with survival and its demonstrated effect on cell proliferation make it a potential prognostic marker and therapeutic target in lung cancer.

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Kelsie Thu

Abstract # 56

FREQUENT DNA DELETION OF miRNA-101, A CANDIDATE TUMOUR SUPPRESSOR, IN LUNG CANCER

Background/Objectives

Tumour suppressor gene (TSG) inactivation is a frequent event in lung cancer that can lead to cellular transformation. DNA deletions can result from selection against TSGs during tumourigenesis, making these genetic events markers for TSG discovery. The objective of this study was to discover miRNAs with novel tumour suppressor functions that are disrupted by DNA deletion in non-small cell lung cancer (NSCLC).

Methods

DNA copy number profiles for 43 NSCLC cell lines and 261 NSCLC tumours, generated by single nucleotide polymorphism and comparative genomic hybridization arrays, respectively, were investigated to determine the frequency of loss for all miRNAs annotated in RefSeq's Hg18 human genome build. Publically available miRNA/mRNA expression profiles for 39/44 cell lines were obtained to determine which miRNAs had strong associations between DNA deletion and miRNA expression, and of those miRNAs, which had expression levels negatively correlated with target mRNA expression.

Results

In total, 105 miRNAs were present in frequent (>40%) regions of deletion, seven of which were strongly associated with gene expression ($p < 0.05$). While our analysis identified the well known TSG miR-let-7, one of the most frequently lost miRNAs was miR-101. miR-101 is transcribed from chromosomes 1p and 9p and is a candidate TSG in multiple cancer types. Considering both genomic locations, miR-101 was lost in 70% of cell lines and 40% of tumours. Analysis of mRNA expression levels for miR-101 target genes, predicted by the miRanda database, in 700 cancer cell lines revealed that MAP2K1 expression was negatively correlated with miR-101 expression ($p < 0.05$).

Conclusion

miR-101 has a strikingly high frequency of DNA loss in lung cancer, suggesting it may be an important tumour suppressor targeted for deletion. miR-101 expression is negatively correlated with MAP2K1 mRNA expression, a key gene involved in the RAS-RAF-MAP signaling cascade which promotes cell growth and survival. These findings suggest miR-101 loss may be a novel mechanism of aberrant RAS-RAF-MAP pathway activation in lung cancer.



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THE ROLE OF EUKARYOTIC TRANSLATION INITIATION FACTOR 2 ALPHA RELATED PATHWAYS IN AUTOPHAGOSOME FORMATION DURING COXSACKIEVIRUS B₃ INDUCED MYOCARDITIS

Background/Objectives

Coxsackievirus B₃ (CVB₃) is one of the predominant strains of viruses causing myocarditis, which is the inflammation of the cardiac muscles. Such inflammation can reduce the ability of the heart to pump blood and may lead to dilated cardiomyopathy, a potentially lethal disorder with few treatment options. Autophagy is a cellular process by which organelles and proteins are isolated by double membrane vesicles (autophagosomes) and degraded following fusion with lysosomes. Previous studies have shown that CVB₃ is able to up-regulate the formation of autophagosomes and utilize these double-membrane structures for more effective replication of viruses. In this study, we aim to: (1) identify the host signalling pathways which are helping in the formation of autophagosomes through eIF2alpha; (2) examine the role of autophagy in CVB₃-induced myocarditis using an autophagy defective transgenic mouse model. It has been reported that CVB₃ infection leads to an increase in the phosphorylation of eukaryotic initiation factor 2 alpha (eIF2alpha), a protein found to positively regulate pathways controlling autophagy. Two notable upstream kinases of eIF2alpha are: PRKR-like endoplasmic reticulum kinase (PERK) found to be activated under endoplasmic reticulum stress and Protein kinase RNA-activated (PKR) activated upon detection of double stranded RNA in cells.

Methods

Following CVB₃ or Mock infection of Human Embryonic Kidney 293A (HEK293A) cells for 4, 8, and 12 hours, cell lysates were collected for Western blot analysis of the phosphorylation status of PERK, PKR, eIF2alpha. Short interfering RNAs (siRNAs) targeting against PERK, PKR and eIF2alpha were used to determine the effects of blocking the pathways on autophagosome formation. Mice with GFP labeled microtubule-associated protein 1 light chain 3 (GFP-LC3), a marker of autophagosome formation, will be used to monitor the effect of CVB₃ infection of mice on autophagy. Moreover, mice with tamoxifen inducible cardiac specific knockout of autophagy related gene 7 (ATG7) will be generated to examine the effect of preventing autophagosome formation on CVB₃-induced myocarditis.

Results

Starting from 4 hours post-CVB₃ infection, an increase in the phosphorylation of PERK relative to mock infection, was observed. eIF2alpha phosphorylation was found to be increased after 8 hours of viral infection and further increased at 12 hours post-infection. PKR phosphorylation levels remained constant during the infection time points; however total protein levels of PKR were decreased after 12 hours of viral infection. To generate cardiac-specific ATG7 conditional knockout mice, we crossed ATG7 floxed-allele mice with inducible cardiac-specific Cre mice from which we obtained progeny for further breeding. GFP-LC3 mice were successfully mated to generate offspring.

Conclusion

Phosphorylation of PERK was increased following CVB₃ infection, which may play a role in CVB₃-induced autophagosome formation via activating eIF2alpha. We are in the process of generating the cardiac-specific ATG7 knockout mice as well as the GFP-LC3 mice to be used for experimental purposes.

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Liat Apel-Sarid

Abstract # 58

AVITENE-INDUCED NECROTIZING GRANULOMATOUS INFLAMMATION DEVELOPING POST-CRANIOTOMY: A PEDIATRIC CASE SERIES

Background/Objectives

Avitene or microfibrillar collagen hemostat (MCH) is a partially water insoluble acid salt of purified bovine corium collagen processed into microcrystals. It is widely used as hemostat agent by surgeons in many surgical fields including neurosurgery. Although considered as effective in creating homeostasis, there are several reports of its adverse effect. Reports about reactions mimicking recurrence of tumor or abscesses are described with every body region. They are also few case reports of Avitene reaction after neurosurgery mimicking recurrence of the tumor, acute disseminated encephalomyelitis (ADEM) or brain abscess.

We are presenting a series of three neurosurgical pediatric cases with characteristic reaction to Avitene, mimicking recurrence or residual of the tumor or abscess formation.

The first case was a 10 years old girl with ganglioglioma of the temporal lobe. At operation Avitene was applied. Six weeks after the procedure she had recurrent seizures and CT scan showed enhancement along the former surgical site. She underwent second surgery and pathological examination revealed granulomatous foreign body reaction to Avitene.

Second case was of a 9 years old boy with anaplastic ependymoma of the left parietal lobe. Five weeks after applying Avitene in the first surgery and 12 radiotherapy treatments he presented with seizures. CT scan showed markedly ring enhancing lesion at the site of the previous resection cavity with perilesion edema. Craniotomy was performed and pathology examination revealed chronic inflammatory and foreign body reaction with residual Avitene.

Third case is of 15 years old girl with occipital cortical dysplasia. Five weeks after the initial procedure, and use of Avitene, she underwent two more surgeries due to ongoing seizures and MRI suggesting of residual cortical dysplasia. Histological examination showed necrotizing granulomatous inflammation and residual Avitene.

These three cases have characteristic imaging and clinical picture 4 to 6 weeks following the use of Avitene in pediatric neurosurgery. It is important that clinicians as well as pathologists consider this characteristic reaction in their differential diagnosis.



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UNIVERSITY OF BRITISH COLUMBIA MICROBIOLOGY CURRICULUM IMPROVEMENT REPORT

Background/Objectives

The Foundations of Medicine course is delivered to University of British Columbia (UBC) medical students in their pre-clinical years. This course is divided into twelve blocks of varying duration. The Host, Defenses and Infection (HDI) block constitutes the Microbiology curriculum and is taught over five weeks. The goal of this project was to improve the HDI curriculum delivered to UBC medical students.

Methods

The project goal was addressed in three ways:

Curriculum comparison: The format and content of the UBC Microbiology curriculum was compared to that of ten North American medical schools using the Curriculum Management and Information Tool (CurrMIT).

Surveys: General practitioners, infectious disease and medical microbiology specialists, and medical students were surveyed to obtain their feedback about curriculum structure and content.

Interviews: Block chairs of the UBC Foundations of Medicine course were interviewed to gain an understanding of the microbiology-related objectives relevant to other blocks and identify areas that were not being addressed.

Results and Conclusions

Curriculum comparison: When compared to other schools that use the block format of curriculum delivery, UBC's course duration (7 weeks) closely matches that of the other schools. Course content analysis revealed areas of curriculum that were covered in the majority of schools used in the comparison but not expressly covered in the UBC syllabus. These included didactic sessions on pathogenic anaerobes, sexually transmitted infections and intra-abdominal infections.

Surveys: All populations surveyed responded that the most effective curriculum structure was as (1) an introductory unit that establishes core principles followed by (2) an organ system-based delivery over 18 months. A combination of the taxonomic and systems based classification was viewed as the most effective strategy for delivery of course content by all populations surveyed.

Interviews: Discussion with the Cardiovascular block chair identified endocarditis as an area needing greater coverage in the HDI curriculum. The Pulmonary block chair identified pneumonia-causing agents and tuberculosis as topics that required shared teaching by the HDI and Pulmonary blocks. Lectures supplemented by laboratory case studies to help students to grasp the basic components of cellular and humoral immunity were recommended by the Blood and Lymphatics block chair.

The suggestions for re-development of the HDI block emerging from this study were presented at a curriculum improvement workshop attended by UBC faculty, with the goal of making positive changes to the current curriculum.

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Jennifer Choo

Abstract # 60

USING TISSUE MICROARRAYS TO DEVELOP AN IMMUNOHISTOCHEMICAL PANEL FOR BASAL-LIKE BREAST CANCER

Background/Objectives

Initially recognized through gene expression analysis, basal-like breast carcinomas are an aggressive subtype of breast cancer that typically portend poor prognosis. Compounded with the fact that we lack effective targeted therapies, they are responsible for a disproportionately high number of breast cancer-related deaths. Identification of basal-like cases in clinical practice is limited by the inaccessibility of gene expression profiling technology to hospital diagnostic labs. However, with some success, studies have attempted to reproduce the expression profile classification of basal-like breast cancers using protein biomarkers predominantly associated via immunohistochemical surrogate definitions. Despite the fact that immunohistochemistry is already commonly used for analysis, researchers in the field have acknowledged that such surrogates lack the sensitivity and/or specificity necessary for an assay to have notable clinical utility in the management of basal-like breast cancer patients, hindering implementation into routine practice. Consequently, while a number of biomarkers show promise for future clinical application - especially as predictive biomarkers to guide therapy - a comprehensive investigation of all proposed biomarkers against a gene expression profile gold standard is necessary as outlined in the present study.

Utilizing a high throughput tissue microarray format, the protein expression of proposed basal-like biomarkers drawn from scientific literature and the latest gene expression profile data was evaluated on breast cancer samples (n = 144) that had previously undergone gold standard subtype assignment. The tissue microarray's high prevalence (31%) of basal-like cases made it an ideal platform to develop the best surrogate immunopanel. For each biomarker, a predefined cutoff was either taken from literature, estimated using a Receiver Operating Characteristic curve or chosen based on universal immunohistochemical scoring schemes. Fisher's Exact test p-values <0.05 were considered to be statistically significant during assessment of biomarker sensitivity and specificity. Corrections for multiple comparisons and statistical model building techniques, such as multivariate logistic regression, were then employed to determine the best subset of biomarkers suitable to comprise the optimized basal-like breast cancer immunohistochemical panel. In turn, the use of this assay to accurately identify basal-like breast cancers in a practical and clinically-accessible manner will enable research to be undertaken in a more meaningful context with regards to basal-like breast carcinoma biology, prognosis, prediction of treatment response and identification of potential molecular targets. Ultimately, the optimized test will allow more personalized treatment options to be made available for the thousands of women afflicted by breast cancer every year, giving them the best chance of a cure.



Suzanne Cheng

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INTEGRIN-INDUCED PROTEIN TYROSINE PHOSPHATASE ALPHA (PTPa) TYROSINE PHOSPHORYLATION REGULATES CAS-MEDIATED CELL MIGRATION

Background/Objectives

Integrins are transmembrane receptors that bind to extracellular matrix (ECM) components to regulate cell adhesion, survival, and migration. The integrin signaling cascade is characterized by a series of protein tyrosine phosphorylation events that are regulated by protein tyrosine kinases (PTKs) and phosphatases (PTPs). Deregulation of these signaling pathways is associated with tumor cell behaviors such as angiogenesis and metastasis. Thus, a precise understanding of integrin signaling mechanisms may lead to the identification of novel molecular targets for anti-cancer therapies. PTPa is a receptor PTP that plays two roles in integrin signaling: It acts proximal to activated integrins to activate the PTK Src, which interacts with focal adhesion kinase (FAK) to initiate multiple downstream signaling events. PTPa is phosphorylated by the active Src-FAK complex on a tyrosine residue in its C-terminal tail, Tyr789, and this is critical for a second undefined role of PTPa in regulating actin stress fiber assembly, focal adhesion formation, and cell migration.

Our objective is to elucidate the role of PTPa-Tyr789 phosphorylation in integrin signaling. We hypothesize that PTPa-phosphoTyr789 functions as a site to recruit integrin signaling molecules involved in cytoskeletal reorganization.

Methods

An *in vitro* model with wild type and PTPa knockout mouse embryonic fibroblasts (MEFs) is adopted to study the molecular mechanisms of PTPa phosphorylation in integrin signaling. We used an adenoviral system to re-express wild type and mutant (Y789F) PTPa in the knockout cells in order to study the function of PTPa-phosphoTyr789. We performed co-immunoprecipitations and immuno-fluorescent staining to identify defective signaling events that are dependent on phosphoPTPa.

Results

We found that tyrosine phosphorylation of the p130Cas (Cas) adaptor protein, a key player in focal adhesion (FA) formation and cell migration, is dependent on integrin-induced PTPa-Tyr789 phosphorylation. The PTK Src catalyzes Cas phosphorylation. In cells expressing mutant PTPa-

Y789F, integrin-induced Src activation is normal but the association of Src with Cas is reduced, indicating that this likely underlies the Cas phosphorylation defect. Since the Cas-Src complex forms in FAs, this suggests that PTPa-phosphoTyr789 may be required for Cas or Src localization to FAs. Our preliminary data indicate that PTPa is localized to FAs in integrin-stimulated cells, supportive of a role for PTPa in potentially regulating the localization of other proteins to these sites. Whether PTPa-Tyr789 phosphorylation is required for FA localization of PTPa, Cas, and/or Src, and the mechanism by which this is effected, is under investigation.

Conclusion

We are delineating a second role of PTPa in integrin-stimulated cell migration. PTPa-Tyr789 phosphorylation is required for the association of Src with Cas and for Cas phosphorylation that is essential for downstream signaling to promote cell migration.

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Edwin S. Gershom

Abstract # 62

HERPESVIRUSES ENHANCE GENERATION OF THE CLOT-DISSOLVING ENZYME, PLASMIN, BY TISSUE PLASMINOGEN ACTIVATOR: CORRELATION TO VIRUS-SURFACE ANNEXIN II

Background/Objectives

Members of the Herpesvirus family, which includes herpes simplex virus type 1 (HSV1) and type 2 (HSV2), and cytomegalovirus (CMV) have been linked as a weak risk factor to vascular disease. When combined with other factors, the impact of viruses contributing as a risk increases significantly. To explain how the virus may be involved, our laboratory has shown that the virus envelope contains anionic phospholipids (aPL) derived from host cells, and proteins encoded by the virus as well as the host genomes, all of which initiate blood coagulation. The current work is based on our additional report that at least one Herpesvirus has host-genome-encoded annexin II on its surface which it uses to enhance infection. Annexin II is known to accelerate tissue plasminogen activator (tPA)-mediated activation of plasminogen to plasmin. The primary physiological role of plasmin is to dissolve clots in a process termed fibrinolysis.

Hypothesis: We hypothesize that Herpesviruses enhance tPA-mediated plasmin generation, which increases cell infection, and this mechanism correlates to the presence of annexin II on the virus.

Methods

Purified HSV1, HSV2 and CMV were propagated in various cell lines and quantified by electron microscopy. Western blots were used to evaluate the presence of annexin II antigen on purified viruses and cells used for propagation. A chromogenic assay was used to evaluate virus mediated-plasminogen conversion to plasmin by tPA using purified proteins. Plasmin-mediated enhancement of cell infection was determined using plaque assays.

Results

Annexin II was demonstrated to be associated with purified HSV1, HSV2 and CMV in all preparations except where cell lines did not express annexin II. Each virus was found to enhance plasmin generation in a dose-dependent manner by up to 5-fold and was dependent on the addition of tPA. The enhancement was diminished for viruses that did not have annexin II. The fold enhancement of Glu -plasminogen (the intact pre-zymogen) was more than Lys-plasminogen (zymogen). Simultaneous incubation of host cells with purified plasmin and viruses enhanced infection by over 3-fold.

Conclusion

Herpesviruses enhance plasmin generation by tPA, and correlates to the presence of viral annexin II. Similar to results with clotting enzymes purified plasmin was shown to enhance infection. Hence, Herpesviruses are capable of activating and exploiting plasma enzymes with opposing clot-forming and clot-busting functions. These data may help to explain why these viruses are not an independent predictor of thrombosis.



Chansonette Harvard

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PHENOTYPIC AND GENOMIC VARIABILITY OF THE 1Q21.1 COPY NUMBER VARIANT (CNV) REGION IN MULTIGENERATIONAL FAMILIES

Background/Objectives

Pathologic chromosomal microdeletions and microduplication (DNA copy number variants or CNVs) detected using whole genome arrays, occur in 10-15% of subject with idiopathic intellectual disability (ID). In addition to pathogenic CNVs array analysis detects CNV with uncertain clinical significance as they occur in both normal and ID subjects and show variable phenotype in subjects with ID. The underlying cause of this phenotypic variability is not known, but several explanations have recently been suggested, including a second genomic hit theory which postulates that a second CNV might be responsible for the variation in the phenotype.

Chromosome 1q21.1 has been identified as a region showing copy number changes resulting in variable phenotype in affected ID subjects, and can also occur in unaffected relatives of the affected subjects. Our current study explores the possible reasons for this variability by a) comparing the 1q21.1 genomic regions deleted or duplicated in affected subjects and their unaffected relatives b) looking for second hit CNV by high resolution whole genome analysis which could contribute to phenotypic variability and c) comparing the whole genome expression profiles for the affected and unaffected subjects. We have noted that in 4 families with 4 subjects with significant ID, 2 mildly affected relatives and 2 phenotypically normal relatives the genomic regions affected did not generally differ significantly between different phenotypic groups. In one family a severely affected female showed an isolated deletion of the 1q21.1 region while her mildly affected brother showed a deletion and a duplication within the same 1q21.1 region. The presence of a second hit CNV was excluded, except in one affected subject with significant ID (this CNV is currently undergoing confirmation). For whole genome expression studies, we have first confirmed expression of genes within the 1q21.1 CNV in the blood from control individuals and then performed whole genome expression studies in affected and unaffected family members using the Illumina array. The expression data analysis is in progress. The results of our work are expected to contribute to the understanding of phenotypic variability of subjects with ID who have the same 1q21.1 CNV and further delineate their genomic and functional correlates.

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Ying Qiao

Abstract # 64

OUTCOME OF ARRAY CGH ANALYSIS FOR 255 SUBJECTS WITH INTELLECTUAL DISABILITY (ID) AND SEARCH FOR CANDIDATE GENES USING BIOINFORMATICS

Background/Objectives

Array CGH enables the detection of pathogenic copy number variants (CNVs) in 5-15% individuals with intellectual disability (ID), making it a promising tool for uncovering ID candidate genes. However, most CNVs encompass multiple genes, making it difficult to identify key disease gene(s) underlying ID etiology.

Methods

Using array CGH we identified 47 previously unreported unique, previously not described, CNVs in 45/255 probands. We prioritized ID candidate genes using 5 bioinformatic gene prioritization web-tools. Gene priority lists were created by comparing integral genes from each CNV from our ID cohort with sets of training genes specific either to ID or randomly selected.

Results

Our findings suggest that different training sets only moderately alter gene prioritization; however only the ID gene training set resulted in significant enrichment of genes with nervous system function (19%) in prioritized versus non-prioritized genes from the same de novo CNVs (7%, $p < 0.05$). This enrichment further increased to 31% when the 5 web tools were used in concert and included genes within mitogen activated protein kinase (MAPK) and neuroactive ligand-receptor interactions pathways.

Conclusion

Gene prioritization web tools enrich for genes with relevant function in ID and more readily facilitate the selection of ID candidate genes for functional studies, particularly for large CNVs.



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EFFECT OF IRON ON ANTIGEN PRESENTING CELLS: IMPLICATIONS FOR TRANSFUSION DEPENDENT HEMOGLOBINOPATHIES

Background/Objectives

The thalassemias and Sickle Cell Disease (SCD) are characterized by destabilized hemoglobin that can lead to a potentially life-threatening anemia. This anemia arises due to iron-driven destruction of the RBC. Removal of oxidatively damaged RBC in vivo occurs primarily via erythrophagocytosis by the mononuclear phagocytic system (MPS). This clearance mechanism may result in negative immune consequences such as the observed increased risk of bacterial infections in these patients.

Methods

To determine the functional consequences of iron and iron chelators on the MPS, the effects of ferric iron (Fe^{3+} ; ferric ammonium citrate, FAC) on dendritic cells (DC) antigen presentation [tetanus toxoid (TT Ag)] and the proliferation of peripheral blood mononuclear cells (PBMC) were examined. The iron chelators tested included Desferal (DFO) and Deferiprone (L1). PBMC were labelled with the fluorescent dye 5,6-carboxylfluorescein diacetate succinimidyl ester (CFSE) to measure cell proliferation. In addition, the effects of iron +/- iron chelators on the expression of CD83, CD80, CD86 and HLA-DR on mature DC were examined.

Results

Importantly, iron significantly inhibited antigen presentation and PBMC proliferation. Treatment of DC cells with 200 μM FAC for 24 hours resulted in a ~70% reduction in PBMC proliferation in response to the TT Ag following 14 days culture. However, inclusion of iron chelators (e.g., 200 μM DFO or L1) restored near normal proliferation. Similarly, CD83 an important co-stimulatory molecule expressed on DC cells was also negatively affected by FAC in a dose (0-200 μM) dependent manner. Following 24 hours treatment with 200 μM FAC, a ~30% reduction in the mean fluorescence of CD83 was observed via flow cytometric assay. Treatment with DFO or L1 overcame the effects of iron on CD83 expression. There were no significant effects of iron on CD80 or CD86.

Conclusion

As shown, iron has significant immunosuppressive effects on antigen presentation and lymphocyte proliferation. Iron chelators can effectively bind and remove free and complexed iron and reverse iron-mediated immunosuppression. These data suggest that iron chelation may provide a mechanism to diminish the risk of recurrent bacterial infections in patients with unstable hemoglobins or with iron-overload (hemochromatosis or secondary iron overload).

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Leslie Chin

Abstract # 66

MECHANICAL PROPERTIES OF ASTHMATIC AIRWAY SMOOTH MUSCLE REVISITED

Background/Objectives

Airway smooth muscle (ASM) is the major downstream effector of the excessive airway narrowing which is characteristic of asthma. Excessive airway narrowing could be related to changes in ASM force, shortening, velocity and/or length adaptability. These properties have been studied in different animal species but data on human tissue are sparse. For example, the force-velocity relationship and length adaptation have yet to be elucidated in human ASM.

Methods

ASM strips from human tracheas were mounted in a myograph capable of measuring force and length simultaneously. Ca²⁺ free-saline solution relaxed the tissue, for estimation of in situ length (L_{ref}). Measurements performed included: force at different muscle lengths (before and after length-adaptation), velocity at different loads (early and late phases of contraction), isometric force recovery after oscillatory length perturbation and maximal shortening from L_{ref}.

Results

Human airway smooth muscle showed significant length adaptation at short and long lengths. Asthmatic ASM demonstrated reduced force adaptation at 50% L_{ref}, increased shortening velocity at low loads, and increased resistance to an oscillatory strain.

Conclusion

The impaired bronchodilating response to deep inspiration seen in asthmatics may be an innate property of the airway smooth muscle itself, independent of the non-muscle components of the airway wall or lung parenchyma.



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Y BOX BINDING PROTEIN-1 IS A MAJOR CONTRIBUTOR TO SARCOMA CELLS MOTILITY AND AGGRESSIVENESS

Background/Objectives

Objectives: 1- Evaluating the effect of YB-1 knock down on sarcoma cells motility (invasion/migration). 2- Detecting the possible relationship between YB-1 and a common marker featuring aggressiveness, hypoxia inducible factor-1 alpha (HIF-1 α).

Background: Sarcoma are diverse group of malignant neoplasms of mesenchymal origin, commonly affecting pediatric age group and are, unfortunately, characterized by early metastatic spread, aggressive behavior and poor prognosis. Genetic alterations play an important role in sarcoma etiology. One of the genes recently believed to play a role in sarcoma is Y-box binding protein-1 (YB-1). Y-box binding protein-1 (YB-1) is a member of highly conservative family of proteins which can bind single and double stranded DNA and single stranded RNA so executing a control over both transcription and translation of multitude of genes. Numerous studies pointed to the essential role of YB-1 in normal development as well as in malignant transformation. One of the well studied models demonstrating the role of YB-1 in carcinogenesis is breast cancer. In this model, it was shown that YB-1 promotes the epithelial-mesenchymal transition (EMT) and hence conferring the malignant cells with the aggressive features. However, very few studies have specifically investigated the role of YB-1 in sarcoma.

Methods

TC32, MG63 and RH30, representing Ewing Family Tumor (EFT), osteosarcoma (OS) and rhabdomyosarcoma (RMS), respectively were selected for the current study. The cells were treated with siCTRL or siYB-1 siRNA from a smart pool. Sarcoma Cell motility was assessed using wound healing assay and Boyden chamber migration assay. YB-1- HIF-1 α relationship was assessed using hypoxia chamber incubation, immunoblotting, polysomal fractionation and real-time RT-PCR.

Results

Interference with YB-1 expression using siRNA from smart pool lead to marked reduction of sarcoma cells motility (Invasion/Migration). Another interesting finding is the intimate relation between YB-1-hypoxia- and HIF-1 . We found that, both of YB-1 and HIF-1 α are induced under hypoxia. Using polysomal fractionation and qPCR, we found that YB-1 is a major translational regulator of HIF-1 α . These findings may explain the role of YB-1 in promoting the aggressiveness of sarcoma cells.

Conclusion

1-YB-1 strongly promotes the migration and invasion of sarcoma cells. 2- YB-1 is a major regulator of HIF-1 α . 3- Both of YB-1 and HIF-1 α are upregulated by hypoxia. 4- YB-1 is very likely a step forward in understanding the aggressive nature of sarcoma and may represent a promising future target in treatment of this type of malignancies.

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Billie Velapatiño

Abstract # 68

THE ACTIVATION OF THE PHOSPHATIDYL-INOSITOL-3 KINASE PATHWAY IN RESPONSE TO THE *BURKHOLDERIA CEPACIA* COMPLEX INFECTION

Background/Objectives

Cystic fibrosis (CF) is the most common fatal inherited disease in North America and bacterial infection is the leading cause of death in these patients. Infections with the Burkholderia cepacia complex (Bcc) can result in rapid decline in lung function and death. Within the species of the Bcc, *B. cenocepacia* (Bc) causes more severe infections than *B. multivorans* (Bm), however the mechanism behind this difference in virulence remains undetermined. We investigated the signaling events, specifically in the phosphoinositide3-kinase (PI3K) signaling pathway after bacterial-host interaction to determine if measures of cellular activation are associated with the differential virulence and persistence of these bacteria in infected host cells in CF patients.

Methods

CF bronchial epithelial cell line (IB3-1), THP-1 derived macrophages and human monocyte-derived MΦs were infected with Bc and Bm. Lysates from infected and uninfected cells were analyzed by Western blot or by immunoprecipitation using the appropriate antibodies.

Results

The activation of the PI3K pathway occurred in IB3-1, in THP-1 derived macrophages and in human monocyte-derived macrophages after infection with live Bc. PI3K was not activated by Bc-LPS in IB3-1 cells but only with live Bc, and this activation was seen up to 3 hours after bacterial challenge. PI3K activation was faster in Bm (5 min) than Bc (30 min) after infection in macrophages. Activation of PI3K was abolished after infection with PI3K inhibitors. The release of TNF-α in macrophages infected with Bc and Bm in the presence of PI3K inhibitor was decreased.

Conclusion

The PI3K signaling pathway is activated in response to the Bcc in both macrophages and epithelial cells. Bm showed a more rapid phosphorylation after infection as compare to Bc in macrophages. Current studies are in progress to correlate the rate of PI3K phosphorylation with cell death via apoptosis or necrosis assays.

This work was supported by a grant from the Canadian Cystic Fibrosis Foundation (to D.P.S.).



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EVALUATION OF TWO CHROMOGENIC MEDIA FOR SCREENING OF GROUP B *STREPTOCOCCUS* IN PREGNANT WOMEN

Background/Objectives

To compare the performance of StrepB Select (SBB) (Bio-Rad) and chromID Strepto B agar (STRB) (BioMerieux) media to that of our routine method for group B Streptococcus (GBS) detection in women at 35 to 37 weeks gestation.

Methods

Five hundred consecutive vaginal/anal swabs were submitted directly to our microbiology laboratory for routine GBS screen between January and July 2009. The swabs were immediately emulsified in 0.5 ml of sterile saline and processed as follows:

- *Routine*: Blood agar plate (BAP) (PML) + Group B Strep (GBS) Broth (PML).

The BAPs and the GBS broths were inoculated, then incubated at 35°C for 18-24 hours. BAPs were examined for colonies with typical GBS morphology. Negative BAPs were re-incubated for an additional 24 hrs and the corresponding GBS broths were sub-cultured to BAPs. All suspect colonies were confirmed by Prolex Streptococcal grouping latex reagents (Group B) (Pro-Lab Diagnostics).

- *Study*: SBB + STRB + GBS broth.

The SBB, STRB plates and GBS broths were incubated for 24 hours at 35°C, then processed as follows: all plates were examined for the presence of GBS colonies (SBB: blue colonies; STRB: pale pink/red colonies) followed by latex agglutination confirmation. Negative SBB/STRB plates were re-incubated for an additional 24 hours and the GBS broth was sub-cultured to SBB and STRB. Confirmation by latex agglutination was considered the gold standard.

Results

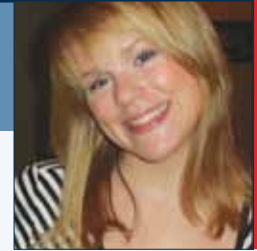
A total of 109/500 samples (22%) were positive for GBS as follows: routine method detected 88/109 (80%); SBB detected 90/109 (83%) at 24 hours, 99/109 (91%) at 48 hrs, 107/109 (98%) after selective broth culture; STRB detected 85/109 (78%) at 24 hrs, 95/109 (87%) at 48 hours, 104/109 (95%) after selective broth culture.

Conclusion

Both chromogenic media were more sensitive than our routine method for detection of GBS. The SBB was easier to read and more sensitive whilst the STRB was more specific after direct reading. However, the specificity becomes less of an issue if all suspect colonies are confirmed by latex agglutination.

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Sophie Stukas

Abstract # 70

THE ROLE OF BRAIN HIGH DENSITY LIPOPROTEINS IN FACILITATING BETA-AMYLOID DEGRADATION

Background/Objectives

A leading hypothesis for the pathogenesis of Alzheimer's disease (AD), is that age-related defects in the degradation and clearance of Beta-amyloid (ABeta) peptides triggers many of the neurotoxic events in AD, including the formation of amyloid plaques. Lipids are transported within the central nervous system (CNS) on apolipoprotein E (apoE)-containing high-density lipoproteins (HDL). Currently, genetic variation in apoE is the only validated risk factor for development of late onset AD. It is known that apoE binds ABeta and alters ABeta metabolism in AD, but the precise mechanisms are not fully understood yet. We have previously shown that loss of the ATP binding cassette transporter A1 (ABCA1), which is responsible for the transfer of lipids onto brain apoE, leads to poorly lipidated apoE and increases amyloid deposition, whereas selective over-expression of ABCA1 virtually eliminates amyloid deposits in mouse models of AD. Furthermore, treatment of AD mice with small molecule Liver-X-Receptor (LXR) agonists, which stimulate ABCA1 and apoE expression, improve cognitive function and decrease brain ABeta levels. However, LXR agonists are not currently safe for human use. We hypothesize that reconstituted HDL (rHDL) can be formulated to mimic endogenous lipidated lipoproteins and function to facilitate ABeta degradation.

Methods

BV2 microglia were treated with 1ug/mL ABeta1-42 and 20ug/mL of various rHDL formulations, with or without 1uM GW3965, an LXR agonist, in serum-free media. After 3h, media was removed, cells were washed thoroughly with PBS and lysed with ice-cold RIPA buffer, sonicated briefly, and harvested by centrifugation at 12,500 rpm for 15min at 40C. ABeta1-42 levels were determined by ELISA (Biosource). Levels of ABCA1, apoE, rHDL, and lipoprotein receptors were determined by western blot. Toxicity was assessed by measuring levels of lactate dehydrogenase in the media by commercial kit (Sigma). rHDL were provided by the Oda laboratory (CHORI) and GW3965 was kindly provided by Dr. John Collins (GlaxoSmithKline).

Results

Additional of exogenous rHDL decreases intracellular ABeta1-42 levels by at least 50% ($p < 0.001$), an effect that is enhanced by co-treatment with GW3965. Smaller, more efflux competent, particles may be more superior in stimulating degradation compared to larger particles. Further, mutated rHDL that cannot accept lipids from ABCA1 or that cannot undergo particle maturation fail to promote ABeta degradation.

Conclusion

These results demonstrate that exogenously added lipoprotein particle can enhance ABeta degradation, and that their capacity to facilitate this process is influenced by their lipidation status. This data suggest that brain HDL may be used as a therapeutic approach for AD by facilitating the degradation of toxic ABeta peptides, without inducing the detrimental side-effects observed with current small molecule LXR agonists.



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COXSACKIEVIRUS B3-INDUCED DOWNREGULATION OF p58IPK IS INVOLVED IN SHIFTING ENDOPLASMIC RETICULUM STRESS TO CELL APOPTOSIS

Background/Objectives

Unfolded (or misfolded) protein responses (UPR) are linked to occurrences of many pathological disorders, such as neuron degeneration, cystic fibrosis, diet-induced obesity, type 2 diabetes and virus-induced diseases. Coxsackievirus B3 (CVB3) is a common pathogen of viral myocarditis in children and young adolescents and its infection can trigger endoplasmic reticulum (ER) stress-mediated UPR. p58IPK is one of the signaling molecules that are involved in the UPR pathway. It is a negative regulator of eIF2 α signaling in cellular mRNA translation process. Although the interactions between p58IPK and other signaling proteins have been determined in previous studies, the functional role of p58IPK during CVB3 infection has not been examined. Our previous investigations have demonstrated that CVB3 infection induced cell apoptosis and also decreased production of p58IPK. However, the connection of these two events has not been elucidated.

Methods and Results

In this study, HeLa cells are either stably transfected with a p58IPK plasmid to over-express p58IPK or treated with specific siRNAs to silence the p58IPK expression. These cells are then infected with CVB3 at a MOI of 10 and used to perform a set of analyses after incubation for different periods of time. Based on the MTS cell viability assay and detection of activation of caspase-3, p58IPK-transfected HeLa cells showed a higher survival rate and lower production of activated caspase-3 than the empty vector-transfected control cells after CVB3 infection, which suggest that p58IPK plays a role in pro-survival and anti-apoptosis process. Moreover, Western Blot analysis of these cell lysates showed a higher expression level of viral protein VP1 and more production of CVB3 particles in p58IPK expressing cells than in the control cells. In contrast, silencing of p58IPK by specific siRNA transfection decreased cell survival rate, sensitized these cells to CVB3-induced apoptosis, and eventually led to a lower expression level of VP1 as compared to the scrambled siRNA-transfected control cells as well as the non-transfected HeLa cells. Since CVB3 infection of non-transfected HeLa cells also downregulated p58IPK expression and up-regulated ER stress marker, the glucose regulated protein 78 (GRP78), our data indicates that persistent CVB3 infection will eventually induce cell death.

Conclusion

p58IPK, as an ER co-chaperone, plays an important role in pro-survival and anti-apoptosis. During CVB3 infection, the virus-induced ER stress responses lead to the downregulation of p58IPK expression and thus result in the transition from pathway of pro-survival to pro-apoptosis.

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Abstract # 72

EFFECT OF POLYMER SPECIES AND SIZE ON IMMUNOCAMOUFLAGE AND ANTIGENICITY

Background/Objectives

Despite ABO/RhD typing, alloimmunization remain significant problem in chronically transfused patients. These problems arise due to the ~300 non-ABO blood group antigens present on the RBC. To address this risk, the immunocamouflage of donor RBC, accomplished by the covalent grafting of low immunogenicity polymers to the cell membrane, has been proposed. To date, the majority of our research has focused on methoxypoly(ethylene glycol) [mPEG]. These studies demonstrated significant immunoprotection and normal in vivo survival. However, a small number of studies have reported the presence of anti-mPEG antibodies in patients receiving PEGylated proteins. We propose to explore the potential utility of a newly designed class of proprietary compounds (cwXXy: cw represent the novel polymer, XX the molecular weight and y the linker chemistry). This novel compound class shares similarities with mPEG, but does not cross react with anti-PEG antibodies. We hypothesize that these polymers (initially exemplified by cw20t and cw30t) will provide similar efficacy to traditional PEGylation, while exhibiting a lower risk of immunogenicity.

Methods

Initial studies were conducted to examine the differential effects of SVAmPEG and cwXXy on RBC structure and function. Normal human erythrocytes (12% hematocrit) were modified with either SVAmPEG or cwXXy polymers (2, 5, 20 and 30 kDa) at concentrations ranging from 0-2 mM. Grafting reactions were carried out for 60 min. Cell morphology was examined microscopically following fixation of control and modified RBC in 4% paraformaldehyde. To assess RBC membrane stability and spontaneous lysis, osmotic fragility assays was performed over 68 hours using Drabkin's reagent. Two-phase partitioning experiments (PEG8000 and dextran T500) were conducted to quantitate any differential effects of SVAmPEG and cwXXy on RBC derivatization based the effects of the grafted polymer on surface properties and phase separation.

Results

Both SVAmPEG and cwXXy polymer species demonstrated equivalent cellular morphology to sham treated control RBC immediately following the derivatization process. Cellular stability, as assessed by the osmotic fragility profile, showed minimal differences between SVAmPEG and cwXXy at eqimolar concentrations and equivalent molecular weights as demonstrated by the fragility curves. However, both polymers showed elevated spontaneous lysis (0.9% saline) at 68 hours relative to unmodified cells. Of importance, previous findings demonstrated that SVAmPEG (2mM grafting concentration) modified cells exhibited normal murine in vivo survival despite this slight osmotic fragility profile variation. Grafting of cw20/30t to RBC results in comparable partitioning curves to SVAmPEG (20 and 30 kDa) in the two-phase system. However, SVAmPEG show slightly enhanced reaction kinetics due to its different linker chemistry that exhibits both greater lysine reactivity and a longer hydrolysis half-life.

Conclusion

Our studies suggest that the cwXXy polymer family demonstrate comparable efficacy to SVAmPEG with regards to morphology, partitioning and osmotic fragility profiles. This data suggests that these novel polymer may exhibit similar in vivo survival and immunocamouflage efficacy.



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REGULATION OF CD1D EXPRESSION IN HUMAN B CELLS BY RETINOIC ACID RECEPTOR

Background/Objectives

Professional antigen presenting cells, including B cells, utilize CD1d to present lipids to NKT cells. NKT cells are innate-like lymphocytes that express a characteristic semi-invariant T cell receptor, which recognizes lipids including the potent glycolipid antigen α -galactosylceramide (GalCer). B cells have been shown to present lipid antigens and to recruit cognate NKT cell help for lipid-directed antibody production. Given the newfound contribution of NKT cells to humoral immune responses, we sought to identify the pathways that regulate CD1d expression in B cells.

Methods

B cell CD1d expression was measured by FACS and qRT-PCR. The ability of B cells to present GalCer to co-cultured NKT cells was assessed by monitoring proliferation and cytokine secretion.

Results

Here we report that activated human B cells express lower levels of CD1d, compared to resting human B cells. Previous studies report that, in dendritic cells, PPAR ligands regulate CD1d expression via the induction of retinoic acid synthesis, which in turn directly up-regulates CD1d expression. We found that RAR ligands (ATRA and AM580), but not PPAR ligands, profoundly up-regulate human B cell expression of CD1d in vitro. RAR ligand-treatment of B cells enhances their ability to present GalCer as well as stimulate NKT cell proliferation and activation. Moreover, NKT cells augmented proliferation of co-cultured AM580-treated B cells at lower GalCer concentrations. Higher GalCer concentrations instead promoted NKT cell cytotoxicity significantly reducing B cell numbers.

Conclusion

We propose that RAR is an important physiological regulator of CD1d expression in B cells. These data further provide a candidate mechanism that underpins NKT cell help for naïve B cells. Our data further demonstrate that downregulation of CD1d immediately following B cell activation may be necessary in order to evade NKT cell regulation/killing.

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Joel Montane

Abstract # 74

T REGULATORY CELL MEDIATED PROTECTION OF ALLOGRAFT IN TYPE I DIABETES

Background/Objectives

Type 1 diabetes is an autoimmune disease characterized by destruction of insulin-producing beta cells. Cellular autoimmune processes in type 1 diabetes comprise not only autoreactive T cells but also dysfunctional T regulatory cells, a subpopulation of CD4⁺ T cells that naturally express both CD4 and Foxp3, a transcription factor that regulates development of these cells. In both mice and humans, T regulatory cells suppress the proliferation of and cytokine production by antigen-specific T cells in vitro. Interestingly, in certain cancers, recruitment of T regulatory cells to tumor sites has been shown to suppress tumor-specific T cell immunity and contribute to tumor growth. Tumor cells attract T regulatory cells to foster immune privilege by producing the chemokine CCL22, which mediates trafficking of T regulatory cells to the tumour. Manipulating Treg cell migration with CCL22 may provide a means of preventing islet transplant rejection in autoimmune diabetes.

Methods

We created an adenovirus (Ad-CCL22) in which a mouse CCL22 cDNA is expressed downstream of the CMV promoter. To determine whether CCL22 expression prevents islet allograft rejection, donor B1/6 islets were isolated and transduced overnight with Ad-CCL22 (n = 9) or Ad-lacZ (n = 8). Islets (300 per recipient) were then transplanted into the left renal subcapsular space of age-matched female Balb/c recipients made diabetic by intraperitoneal administration of streptozotocin (STZ). Blood glucose levels were followed in transplant recipients and at various time points post-transplantation the grafts were harvested for histological and FACS analysis.

Results

Isolated islets transduced with Ad-CCL22 (10 MOI) produced and secreted high levels of CCL22 in vitro whereas CCL22 production by non-transduced islets was undetectable. Ad-CCL22 or Ad-lacZ-transduced islets were transplanted into MHC mismatched STZ-diabetic recipients. By 12 weeks post-transplant, only 1 of 8 CCL22-expressing islet grafts had been rejected as indicated by return of hyperglycemia, whereas all Ad-LacZ-transduced grafts were rejected. Animals transduced with Ad-CCL22 expressed high levels of CCL22 and glucose intolerance was not affected. A higher proportion of Treg cells was observed in islet grafts transduced with Ad-CCL22, compared with non-transduced islet grafts by immunostaining for Foxp3 and FACS analysis. The high proportion of Treg cells in CCL22-expressing islet grafts was maintained for up to 60 days post-transplantation.

Conclusion

These findings indicate that induced expression of CCL22 by islets recruits naturally occurring Treg cells to transplanted islets, preventing activation of alloreactive T cells and beta cell destruction and thereby decreasing allograft failure and recurrence of diabetes in this model. Manipulation of Treg cells by CCL22 in transplanted islets may be a novel therapeutic strategy for preventing islet transplant rejection in diabetes.



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SULFATIDE-REACTIVE T CELLS IN MULTIPLE SCLEROSIS

Background/Objectives

Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. The majority of myelin consists of lipids (70 percent). These lipids are presented by antigen presenting cells (APC) via CD1 molecules and recognized by CD1-restricted T cells. Apolipoprotein E (ApoE), a lipoprotein present in cerebrospinal fluid, has been shown to promote capture and presentation of lipids.

Methods

Lipids were isolated from bovine brain myelin using sucrose density ultracentrifugation, chloroform:methanol extraction and purification using an aminopropyl and C18 column. Also, commercially available lipids or synthesized sulfatide were used. Lipid presentation was studied by culturing CD1-restricted T cells with CD1-transfected K562 cells in the presence or absence of ApoE.

Results

From all studied commercially available myelin lipids, only sulfatide showed to induce IFN-gamma production by CD1-restricted T cells. This activity was reduced when adding anti-CD1 blocking antibodies to the culture. Treatment of sulfatide with HCl removed the sulfate group, resulting in galactocerebroside and reduced the reactivity of the lipid. Similar results were found when isolated myelin lipids from bovine brain or synthesized sulfatide were used. The later one also demonstrated enhanced reactivity when cultured in the presence of ApoE.

Conclusion

Sulfatide, one of the most abundant lipids in myelin, is presented to human CD1-restricted T cells and ApoE can enhance this presentation. This implies that sulfatide could be a potential autoantigen in MS. In addition, ApoE may play a role in mediating an immune response against myelin lipids. These findings have important implications for the pathogenesis of MS and the development of new therapeutic applications.

Index

A		
Abozina A.	45	
Abraham T.	55	
Allan L.	32, 84, 86	
Allard M.	29, 36, 37, 38, 39, 40, 44	
Al-Rawahi G.	80	
Al-Riyami A.	11	
Al-Sarraf A.	12, 51	
Anantha M.	42	
Andersen R.	30	
Anderson K.	53	
Andolfatto, G.	14	
Ang L.	29	
Aparicio S.	56	
Apel-Sarid L.	69	
B		
Baker M.	81	
Bally M.	42, 43	
Bashashat A.	26	
Baybik J.	18	
Bell L.	56	
Berean K.	53	
Bischoff L.	85	
Bleackle C.	29	
Boivin W.	28, 29, 55	
Bondy G.	44	
Book L.	80	
Bossé Y.	77	
Brooks D.	62	
Bryce E.	54	
Burgess B.	81	
Byrne A.	69	
C		
Carrier M.	56	
Carter I.	25	
Cetinbas N.	35	
Champagne S.	13	
Chari R.	65, 66, 67	
Chaworth-Musters T.	47	
Cheng J.	60, 71	
Cheng S.	72	
Chi K.	18	
Chin L.	77	
Chipperfield K.	11	
Chiu N.	58	
Choo J.R.	71	
Chow C.	71	
Chow E. 7	4	
Chow K.	56	
Chu F.	46	
Chung B.K.	32, 84	
Clarke B.	16	
Cochrane D.	69	
Constantinescu I.	63	
Côté H.	24, 45, 46, 47, 48	
D		
Dai D.	22	
Dai L.	85	
Daley G.	20	
Dedhar S.	43	
DeValle N.	81	
Devlin A.	49	
Dunham C.	69	
Dunn S.	43	
E		
Einhorn W.	20	
Elliott W.M.	38	
El-Naggar A.	78	
Enfield K.	65	
F		
Fawcett C.	75	
Forbes J.	47	
Frohlich J.	51	
Fung F.	18	
G		
Gadawski I.	24, 48	
Gale N.	56	
Gao D.	17, 71	
Gao G.	62, 68	
Gardy J.	57	
Garmaroudi F.	26	
Garnis C.	52, 53	
Garratt J.	17	
Gazdar A.	65, 66	
Gelmon K.	43	
Gershom E.	73	
Gilks B.	16, 17, 56	
Glier M.	49	
Gosh S.	49	
Grant J.	54	
Granville D.	28, 29, 55	
Guns E.	58	
H		
Hackett T.	77	
Hamilton J.	63	
Hamilton S.	74	
Hancock R.	62	
Haque M.	12	
Hardenberg G.	85	
Harris M.	24, 44	
Harvard C.	74, 75	
Hassal E.	19	
Hemida G.	82	
Hemida M.	27	
Hernandez-Lee. J.	50	
Hiebert P.	55	
Hirsch-Reinshagen V.	81	
Hoang L.	70	
Holden J.	74, 75	
Holmes D.	21	
Hughes S.	52	
Hull M.	24	
Huntsman D.	15, 56, 57	
I		
Imam T.	47, 48	
Innis S.	61	
J		
Janes K.	26, 61	
Jewell K.	57	
Jia W.	58	
Jitratkosol M.	24, 47	
Johns K.	44	
Joseph C.	60	
K		
Kaliciak H.	59	
Kalloger S.	56	
Kalra J.	43	
Keller B.	60, 61	
Kelly N.	70	
Kennett J.	31	
Kindrachuk J.	62	
Kizhakkedathu J.	62, 63, 64	
Kyluik D.	83	
L		
Lam L.	66	
Lam S.	65, 66	
Lam W.	31, 65, 67	
Lapointe N.	47	
Lau S.	71	
Lee A.	15	
Lee Carol	38	
Lee Charles	38	
Leung C.	36, 37, 38, 41	
Leung S.	17, 71	
Levings M.	23, 85	
Lewis M.	74	
Lewis S.	75	
Lichtenstein S.	39	
Liu X.	75	

Liu Z.	27	Pavlidis P.	74, 75	Sun G.	72
Lockwood W.	66	Pazooki S.	55	Sutherland B.	43
Lu F.	16	Pengill S.	13	Sutherland M.	73
Luk M.	56	Pikor L.	66	T	
Luo H.	26, 68	Poh C.	52	Talbot K.	25
M		Poon T.	68	Tan R.	23, 32, 84, 85
Maan E.	48	Potter K.	22	Tang P.	57
MacAulay C.	65	Prabhu K.	70	Taraboanta C.	51
MacGillivray R.	25	Priatel J.	23, 32	Thomas E.	80
MacNeil D.	53	Prydzial E.	25, 73	Thu K.	67
Mak S.	82	Pudek M.	12	Tognon C.	33, 78
Marchant D.	26	Q		Towle R.	53
Marles S.	74	Qiao Y.	74, 75	Trigo G.	33
Martell S.	74	R		Tsui I.	52
Martz W.	59	Rajcan-Separovic E.	74, 75	Turashvili G.	56
Marwaha A.	23	Rees H.	15	Turbin D.	17
Masin D.	42	Ritchie G.	13	Tyson C.	74, 75
Mathers J.	78	Robertson M.	20	U	
Matzke L.	36, 37, 38, 39, 41	Roland K.	11	ul-haq M.	63
Mawji N.	30	Romney M.	13	V	
May S.	81	Rotblat B.	33, 35	van den Elzen P.	32, 84, 86
Mayor T.	35	Ryan P.	16	Vanden Hoek A.	25
McGillivray B.	74	S		Varnals T.	29
McManus B.	26, 29, 36, 38, 41	Sadar M.	30	Velapatiño B.	79
McNeill J.	40	Saeedi R.	44	Verchere B.	22, 85
Meredith A.	41	Samra A.	37, 41	Verreault M.	42
Mhanni A.	74	Samson J.	47	Vickars L.	25
Money D.	47, 48	Saran V.	40, 44	Vucic E.	65, 66
Montan E.J.	85	Sarwal G.	51	W	
Montaner J.	24, 44	Sattha B.	47, 48	Wade R.	24, 46
Montessori V.	24	Schaeffer D.	19	Walker D.	46
Montoya V.	57	Scott M.	76, 83	Wambolt R.	40
Morshed M.	70	Seow C.	77	Wang D.	76
Murphy K.	26	Shackleford M.	28	Waterhouse D.	42
Mustafa i.	76	Shah S.	57	Webb J.	39
N		Sharma V.	40	Wellington C.	81
Ng R.	26	Siow S.	50	Westwell-Roper C.	22
Ng T.	20	Solomon D.	77	Wilkinson A.	81
Ng Y.	56	Sorensen P.	20, 33, 35, 78	Williams D.	30
Nielsen T.	17, 71	Soudeyns H.	47	Williams S.	29
Nietlspach F.	39	Soukhatcheva G.	85	Willman, E.	14
Novak E.	61	Speert D.	79	Wilmer A.	54
O		Spencer H.	46	Wilson I.	65
Oda M.	81	Stax A.	84, 86	Wolber R. 1	7
Oliveira L.	48	Steinbok P.	69	Wong J.	68
Owen D.	15, 19	Stratford A.	43	Wong T.	13
P		Strong E.	74	Y	
Pallen C.	72	Strutt D.	42	Yang D.	27, 82
Panagiotopoulos C.	23	Stuka S.	81	Yang Y.C.	30
Papp E.	48	Sturby T.	19	Yapp D.	42
Paré P.	77	Sulistyoningrum D.	49		

Ye X.	27, 82
Yee S.	31
Yoshida E.	12
You M.	65
Yu K.	64
Yuen V.	40
Z	
Zhan Y.A.	65
Zhang F.	78
Zhang J.	68
Zhang M.	27, 82
Zhao H.	28, 29, 55
Zheng D.	84, 86
Zhu H.	20
Zolein M.	21

NOTES



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