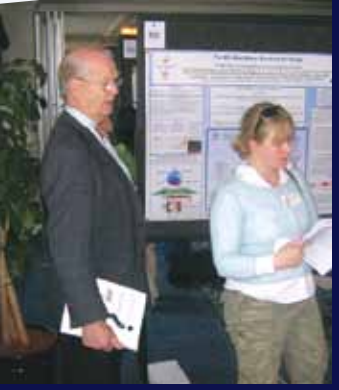

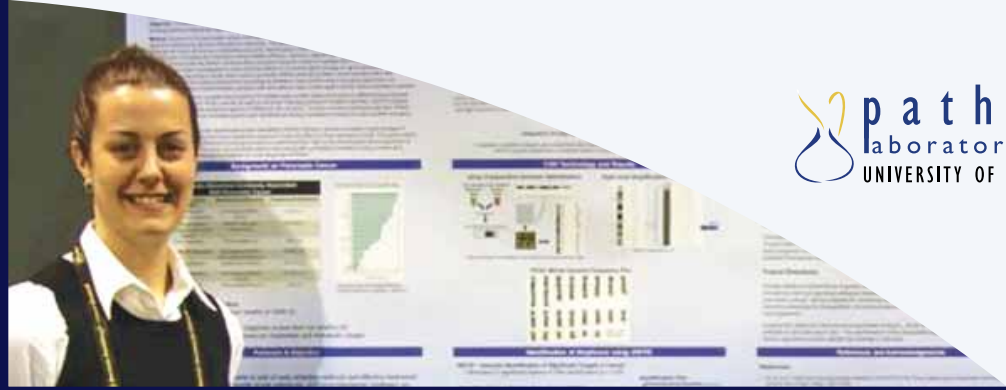

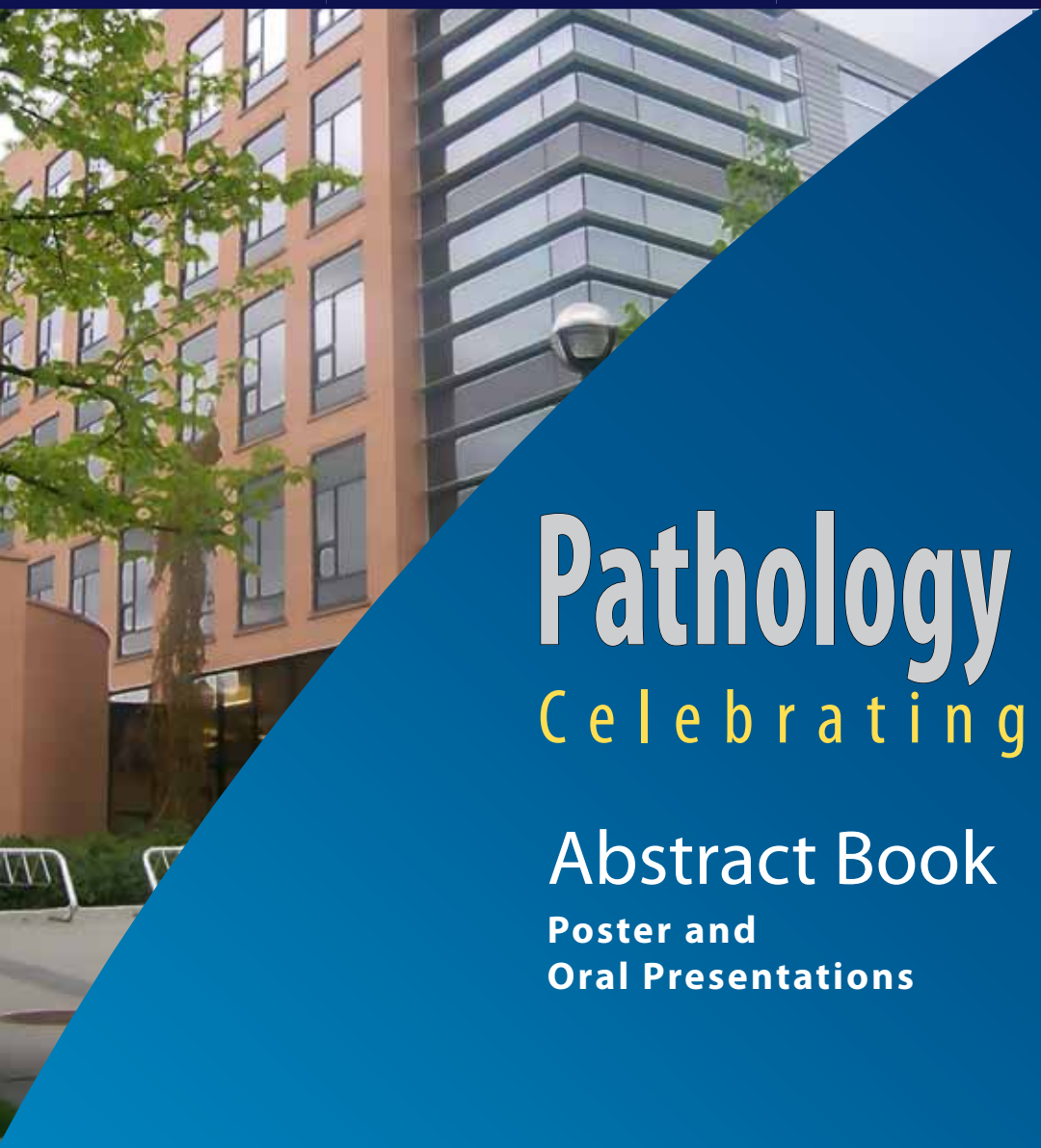

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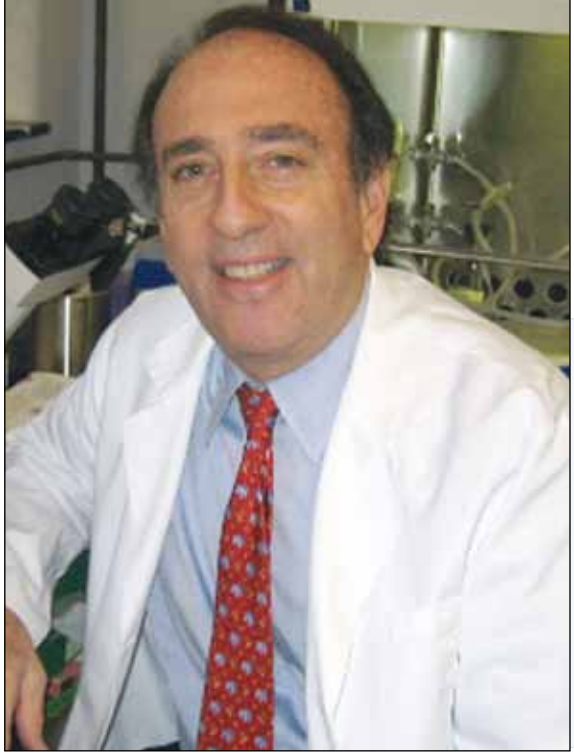
Pathology Day 2009

Celebrating Together

Abstract Book

Poster and
Oral Presentations



Dr. Jay Levy, Professor of Medicine, Univ of California

pathology laboratory medicine
UNIVERSITY OF BRITISH COLUMBIA

UBC

"This year's James Hogg Keynote Lecture will be given by Dr. Jay Levy, Professor of Medicine, University of California, San Francisco "

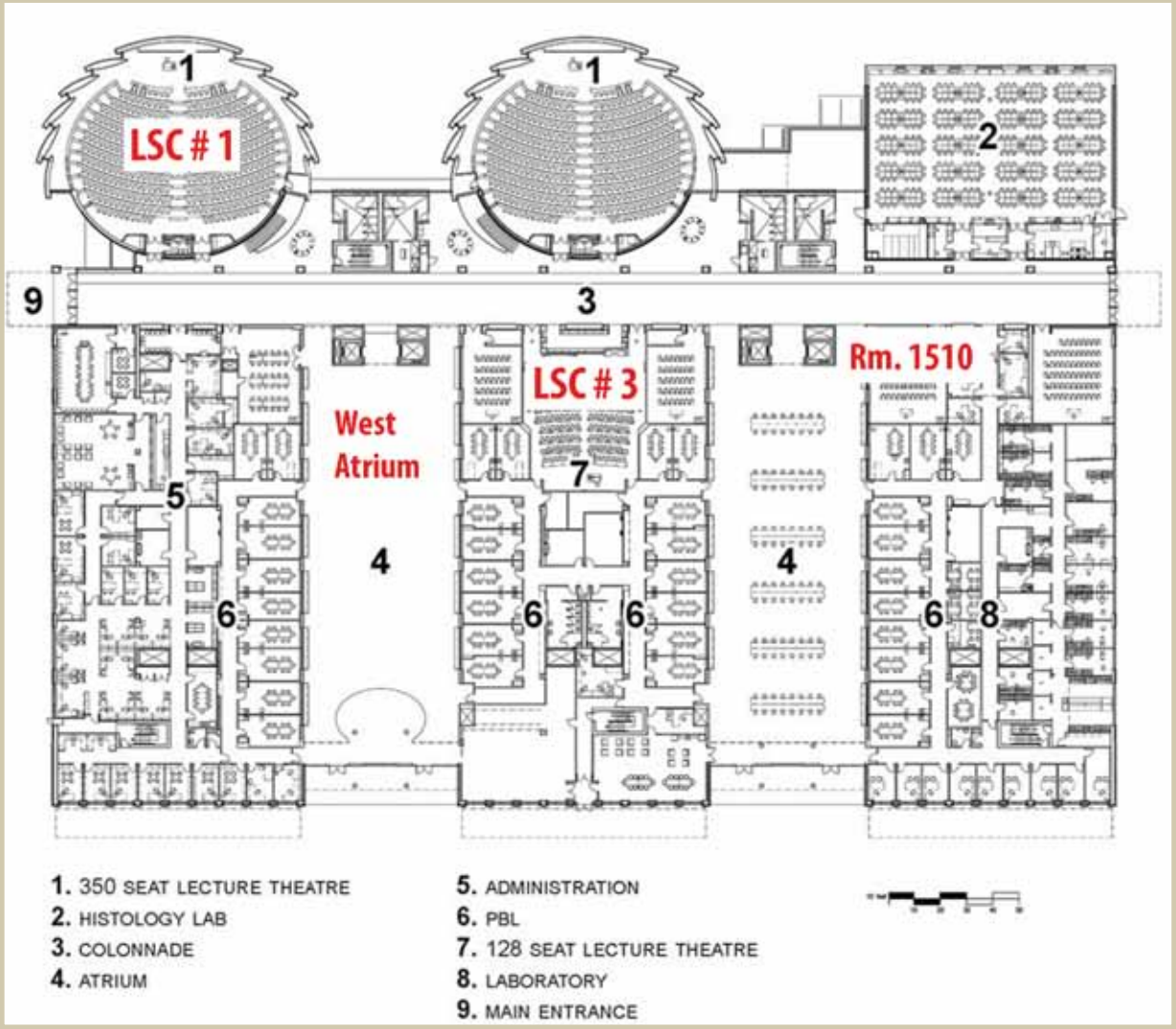
Welcome to The Pathology Day 2009

Education and Research are the reasons for the existence of the University, and this event is a celebration of our departmental research enterprise. It is the primary opportunity for members of our geographically and intellectually far-flung department to get together, socialise, and find out about research taking place at other sites within the UBC system. It also allows us to recognise members of our department who have made outstanding contributions over the previous year or, in the case of the David Hardwick Lifetime Achievement Award, over the course of a career. As befits a celebration of research, where risk-taking can be rewarded (or not), our evening festivities will be at the River Rock Casino for the first time this year. The Department is considering a proposal whereby we would take advantage of this evening at the casino to try to re-build the departmental endowment fund, after the set-backs of 2008. We are extremely pleased to have as this year's James Hogg Keynote Lecturer, Dr. Jay Levy (UCSF); Dr. Levy continues our tradition having world-leaders in biomedical research presenting to our department at Pathology Day and interacting with students, fellows, and faculty. Special thanks to Dr. Hélène Côté for her efforts in organizing this year's festivities.

With warmest best wishes,

Dr. C. Blake Gilks
Professor and Acting Head
Department of Pathology and Laboratory Medicine

LSI Floor Plan



West Atrium: Coffee & Muffins | Lunch | Poster Presentations
LSC # 1: Keynote speaker lecture
LSC # 3: Graduate & Post-doc Oral Presentations
Room 1510: Residents Oral Presentations

Acknowledgment

Special acknowledgement to the sponsorship provided by Roche

Roche

Putting together Pathology Day is always a team effort and I would like to extend special thanks to ***Dr. Blake Gilks***, ***Maureen Barfoot*** and ***Susana Martin*** for their help organizing this event, and to ***Debbie Bertanjoli*** for the preparation of the abstract book. I also wish to extend my gratitude to the department's ***Faculty, Staff and Graduate Students*** who have and will assist with various tasks. Thanks to those who helped review the abstracts and to the many people who will help judge the oral and poster presentations, their contribution is much appreciated. Finally, thanks to the ***department's staff and students*** who will help ensure that all is in place and runs smoothly on may 28th.

Hélène Côté
Chair Pathology Day 2009

Conference Outline

Pathology Day 2009

08:30 Breakfast - LSC West Atrium

Resident Oral Session, Room 1510

abstract #		
	09:00	Opening Remarks by Dr. Diana N. Ionescu
1	09:15	**Fahad Alghamdi** Negative Immunohistochemical Controls (No Primary Antibody): Influence of Different Detection Systems and Antigen Retrieval Techniques on Non-specific Staining
2	09:27	**Arwa Al-Riyami** Paroxysmal Nocturnal Hemoglobinuria in British Columbians: Flow Cytometric Analysis
3	09:39	**Tyler Smith** Molecular Basis for a Thrombophilic Patient's Combined Deficiencies in Proteins C and S
4	09:51	**Ahmad Al-Sarraf** CETP in Rare Dyslipidemias
5	10:03	**Vincent Fung** Re-evaluation of the Correction Factor for Ethanol in the Calculation of the Osmolal Gap
	10:15 - 10:45 BREAK	
6	10:45	**Tony Ng** The Anoikis Stress Response Induces the AMPK Energy-sensing Pathway to Promote Survival in Transformed Cells
7	10:57	**Rola Ali** Non-overlapping Molecular Abnormalities in Endometrial Stromal Neoplasms Compared to Uterine Tumors Resembling Ovarian Sex Cord Tumor (UTROSCT)
8	11:09	**Jefferson Terry** NTRK2 Expression Predicts Improved Survival in Squamous Cell Carcinoma of Lung
9	11:21	**David F. Schaeffer** Insulin-Like Growth Factor-2 mRNA Binding Protein 3 (IGF2BP3) Expression May Be a Marker for a Unfavorable Prognosis in Pancreatic Ductal Adenocarcinoma
10	11:33	**Karen Ung** The Prognostic and Predictive Significance of Biological Classification of Primary Breast Cancer with Tissue Microarray from NCIC CTG MA12 trial
	11:45 - 13:15 LUNCH, (West Atrium)	
	13:15 - 15:15 POSTER PRESENTATIONS, (West Atrium)	
11	15:15	**Anna Lee** FLI-1 is a Specific Marker of Ewing Sarcoma in Non-hematolymphoid Small Round Blue Cell Tumors of Bone
12	15:27	**Fang-I Lu** Longitudinal Study of Sessile Serrated Adenoma of the Colorectum
13	15:39	**Kennard Tan** Mumps Virus Detection by RT-PCR and Culture During an Outbreak in a Predominantly Unvaccinated Population
14	15:51	**Aleksandra Stefanovic** Identification of a Novel Genomic Island in Invasive Streptococcus pneumoniae Serotype 5 Outbreak Strain
15	16:03	**Miguel Imperial** Performance of an Algorithm Using TechLab C. DIFF CHEK and TechLab C. DIFFICILE TOX A/B II as Compared to BD Cdiff GeneOhm PCR for the detection of Clostridium difficile in Stool Samples

Conference Outline

2009

Graduate Students and Post-Doctoral Fellows Oral Session, Room LSC3

abstract #		
	09:00	Opening Remarks by Dr. C. Blake Gilks
16	09:15	**Henry Stringer** Mitochondrial DNA (mtDNA) Depletion and Deletion in Statin-Induced Myopathy
17	09:30	**Tyler Hickey** Identification of Cpn60.2 as a Surface Ligand of *Mycobacterium tuberculosis* that Facilitates Bacterial Association with Macrophages via CD43
18	09:45	**Jerry Wong** The Role of Serum Response Factor in Coxsackieviral Cardiomyopathy
19	10:00	**Marissa Jitratkosol** Mitochondrial DNA (mtDNA) Mutations in Infants Exposed to HAART *in utero*
	10:15 - 10:45 BREAK	
20	10:45	**Cleo Yi-Fang Lee** MicroRNA Regulation of Oncolytic Herpes Simplex Virus Type 1 for Selective Killing of Prostate Cancer Cells
21	11:00	**Darrell Bessette** The role of PRL-3 in Mediating the Effects of p53 in Prostate and Breast Cancer Cell Lines
22	11:15	**Peyman Tavassoli** TAF1, From a General Transcription Factor to Modulator of Androgen Receptor in Prostate Cancer
23	11:30	**Ivy Tsui** DNA Amplification Occurs in Oral Dysplasias and Disrupts Multiple Genes in One Signalling Network
	11:45 - 13:15 LUNCH, (West Atrium)	
	13:15 - 15:15 POSTER PRESENTATIONS, (West Atrium)	
24	15:15	**Agatha Jassem** Outer Membrane Mediated Aminoglycoside Resistance in the Burkholderia Cepacia Complex
25	15:30	**Varun Saran** Control of Glycolysis in Cardiac Hypertrophy: Interaction of Hexokinase and Voltage Dependant Anion Channel
26	15:45	**David Marchant** P38 MAP Kinase is Activated by Toll like Receptor 4 via MyD88 During Virus Entry to Activate Virus Internalisation and Replication
27	16:00	**Sohrab Shah** Mutational Evolution of a Lobular Breast Tumour, Profiled by Whole-transcriptome and Whole-genome Next Generation Sequencing

James Hogg Keynote Lecture, Room LSC # 1

16:30 **Dr. Jay Levy,** University of California, San Francisco
The Global HIV/AIDS Pandemic: How Science Faces the Challenge

Reception, Dinner & Awards Presentations, Ballroom

18:30 River Rock Casino Resort, Fraser Room
8811 River Road Richmond, BC

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Oral Presentations

Fahad Alghamdi[1], Malcolm Hayes[2], James Cupples[3], Erika Mehl[4], Beverley Wolber[1], C Blake Gilks[1]

[1]Dept of Pathology, Vancouver General Hospital, Vancouver, BC, Canada, [2]Dept of Pathology, British Columbia Cancer Agency, Vancouver, BC, Canada, [3]Dept of Pathology, Royal Columbian Hospital, Westminster, BC, Canada

Fahad Alghamdi

Negative immunohistochemical controls (no primary antibody): influence of different detection systems and antigen retrieval techniques on non-specific staining

Backround/Objectives

Immunohistochemisty is a sensitive and specific technique for localisation of proteins in tissue sections. Quality assurance in diagnostic immunohistochemistry is evolving and there are currently no generally accepted standard practices for the use of negative controls. The aim of this study is to assess how variables such as automated immunostainer model, detection system, and antigen retrieval technique influence non-specific staining in no-primary-antibody negative control slides. Our ultimate goal is to determine the optimal use of negative controls in diagnostic immuhistochemistry.

Methods

Unstained slides were cut from tissue microarray constructed from 0.6 mm cores of malignant and normal tissue from 76 cases, including 26 different malignant and 15 normal tissues. These were subjected to different pretreatment protocols including: mild, moderate (standard), and long heat (done on an automated immunostainers), heat antigen retrieval in a pressure cooker and microwave, and enzymatic digestion. Different buffers were used for heat antigen retrieval. The slides were stained in four different laboratories, using different automated immunostainers (Ventana Benchmark XT, Ventana Discovery XT, Bond-Max) and with different commercial detection kits, including avidin-biotin and polymer-based detection systems.

Results

With polymer-based detection systems, all slides showed no staining except for endogenous cytoplasmic or extracelluar pigments, such as lipofuscin, melanin, and hemosiderin. With avidin-biotin detection system, some of the tissues show granular cytoplasimic staining, that ranges from very weak to strong. The positivity and intensity of the staining showed marked differences depending on the antigen retrieval technique used. In the two labs using avidin-biotin detection systems, the staining increased with increasing antigen retrieval (longer heat > shorter heat > protease). A Tris-EDTA buffer at pH 8.0 showed more false positive staining than a citrate buffer at pH 6.0.

Conclusion

Polymer-based detection systems offer superior specificity, with less non-specific staining than avidin-biotin detection systems. The amount of staining (presumably as a result of endogenous biotin) seen with the latter varied with different antigen retrieval systems. Buffers used for heat retrieval also affect background. The highest background is seen with long antigen retrieval in heat, using a Tris-EDTA buffer at pH8.0. Even with a polymer based detection system, endogenous cytoplasmic or extracelluar pigments can be present and the only use of the no-primary-antibody is to detect these pigments, which can be detected with less cost by using a negative control that omits both primary antibody and the detection system. For avidin-biotin detection systems, a single no-primary-antibody negative control with the most aggressive antigen retrieval protocol to be used for that case will suffice to detect endogenous biotin staining.

Abstract # 2

Arwa Al-Riyami

Arwa Z. Al-Riyami, Bakul I. Dalal

Division of Hematopathology, Vancouver General Hospital, Dept Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Paroxysmal nocturnal hemoglobinuria in british columbians: flow cytometric analysis

Backround/Objectives

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired disorder of the pluripotent stem cells characterized by partial (type II) or complete (type III) deficiency of glycosyl phosphatidyl inositol anchored proteins (GPI-APs). Clinically, PNH may present either as episodic intravascular hemolysis or recurrent thromboses in unusual sites or with cytopenias. Subtype of PNH and its clone size predict the clinical presentation and response to newer biological treatments. In the past decade, flow cytometry has replaced the sugar water lysis and Ham's tests as preferred diagnostic tool. Besides diagnosis of PNH, flow cytometry is also useful for predicting clinical presentation and response to treatment. However, criteria for PNH diagnosis, its subtype and clone-size determination have not been clearly defined. In this retrospective study we reviewed the flow cytometric profile of PNH patients diagnosed in British Columbia since the implementation of this test in 2001. We compared the usefulness of different GPI-APs in detecting PNH clones, determining the clone size and type, and in predicting the risk of thrombosis.

Methods

Clinical and flow cytometric data on PNH patients diagnosed between January 2001 and March 2009 were reviewed. Flow cytometric testing for PNH in our laboratory includes assessment of GPI-APs CD55, CD59 and Fluorescent Pseudomonas aeruginosa toxin (FLAER). The diagnostic criterion for PNH in our laboratory is based on the absence of at least two GPI-APs in two hematopoietic cell types. PNH subtype and clone size as determined by different GPI-APs expression in different cell types were compared. Clinical presentation (hemolysis / thrombophilia / cytopenias) were correlated with subtype of PNH and clone size.

Results

17/417 patients had PNH (median age: 39 years, range 10-79 years). Clinical features included cytopenias (n=16, 94%), hemolysis (n=13, 76%) and thrombosis (n=4, 24%). There was significant variability in expression of GPI-APs CD55, CD59 and FLAER in different cells types. Granulocyte expression of CD55 and FLAER and monocyte expression of CD55 were most sensitive in diagnosing PNH. Size of the PNH clone was consistently larger for the granulocytes and monocytes. Cut off of 60% PNH clone size as determined by CD55 analysis for granulocytes and monocytes correlated with thrombotic events (P=0.0192), suggesting prophylactic anticoagulation in these patients. Subtyping of PNH was variable depending on the GPI-AP and cell types analyzed. Determination of PNH subtype II (reduced GPI-APs) tended to be subjective, based on the mean channel of fluorescence (MCF). We propose using a ratio of MCF of CD55 or CD59 expression of normal and PNH granulocytes for determination of PNH subtype (type II >0.1, type III <0.1).

Conclusion

Expression of GPI-APs on different blood cells is variable. Granulocyte CD55 and FLAER and monocyte expression of CD55 are most sensitive for PNH diagnosis. Granulocyte and monocyte CD55 expression was best in predicting thrombotic events. Ratio of CD55, CD59 expression of normal and PNH granulocytes improves determination of PNH subtype. There was no correlation of the flow cytometric profile with hemolysis or cytopenias.

Smith TW[1], Carter ISR[2], Carter CJ[1,2], and MacGillivray RTA[2]

[1]Dept of Pathology and Laboratory Medicine; [2]Centre for Blood Research and Dept of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada

Tyler Smith

Molecular basis for a thrombophilic patient's combined deficiencies in proteins C and S

Backround/Objectives

Proteins C and S (PC and PS) are vitamin K-dependent plasma proteins with anticoagulant properties. Protein S functions as a non-enzymatic cofactor for activated protein C (APC). APC proteolytically degrades coagulation factors Va and VIIIa, thereby diminishing the activities of prothrombinase and tenase complexes, respectively. The human PC gene (PROC) is located on the long arm of chromosome 2 (2q13-q14) and contains 9 exons which code for 461 amino acid residues. The human PS (PROS1) gene resides on chromosome 3 (3p11.1-q11.2) and contains 15 exons coding for 636 amino acid residues. Hereditary PS and PC deficiencies are both autosomal dominant disorders in which patients have diminished functional levels of the respective protein (approximately 50% relative to normal controls). Clinically, this results in increased propensity toward thromboembolic disease, also known as thrombophilia. In this study, we describe a unique thrombophilic patient who has combined deficiencies in both proteins C and S. The objective of the study was to elucidate the precise genetic defect(s) causing these deficiencies.

Methods

Following purification of the patient's DNA from peripheral blood leukocytes, PCR amplifications were performed using oligonucleotide primers flanking all exons of the PROS1 and PROC genes. In addition, the 400bp region upstream of the first exon of PROS1 (corresponding to the promoter region) was also PCR amplified. The PCR products were purified, then sequenced in both forward and reverse directions using the dye-terminator method. The resultant nucleotide sequences were visually inspected for possible heterozygous mutations and compared with the PROS1 and PROC reference sequences.

Results

The patient was found to be heterozygous for a missense PROC gene mutation in exon 8, which in part codes for the proteolytic domain of protein C. The resulting valine-to-glycine amino acid substitution of residue 221 is in close proximity to the catalytic triad, which could abrogate its enzymatic activity. Although no mutations were present in any of the patient's PROS1 exons, there was a heterozygous C-to-G nucleotide substitution in the PROS1 promoter region. This substitution is present within an Sp1 transcription factor binding site that is highly conserved among mammals. Therefore, this mutation could significantly diminish expression of the otherwise normal PROS1 gene, leading to the observed decreased protein S level. Both mutations are novel, as they are unreported in the literature and are not listed in the PROC and PROS1 mutation databases.

Conclusion

We have successfully identified two novel genetic defects leading to deficiencies of the anticoagulant proteins C and S in a patient with thrombophilia.

Abstract # 54

Ahmad Al-Sarraf

A. Al-Sarraf[1], K. Sachs-Barrable[2], J. Hill1, K. Wasan[2], J. Frohlich[1]

[1]Healthy Heart Program, St Paul's Hospital, [2]Genetic Pathology Evaluation Centre, Vancouver, BC, Canada

CETP in rare dyslipidemias

Backround/Objectives

High-density lipoprotein (HDL) particles accept free cholesterol from peripheral cells. It is then converted into cholesteryl ester (CE) by lecithin:cholesterol acyltransferase (LCAT). HDL-CE can be then transferred to triglyceride-rich lipoproteins via CE transfer protein (CETP).

CETP is important for remodeling of HDL particles, transferring CE from HDL to TG-rich lipoproteins in exchange for TG. This leads to the formation of HDL particles that are depleted of CE and enriched in TG. These particles are the preferred substrate for hepatic lipase which hydrolyses the TG leading to the formation of small, dense HDL particles. Low levels of high-density lipoprotein cholesterol (hypoalphalipoproteinemia (HA)) result from a variety of conditions ranging from secondary causes such as smoking, obesity and DM type 2 to specific genetic mutations such as Tangier disease (TD), LCAT deficiency and fish-eye disease (FED). In LCAT deficiency and FED, the risk of atherosclerosis is not increased, while there is a slight increase in the risk of coronary heart disease (CHD) in TD. **Objective**. To determine CETP mass and activity in rare disorders of HDL metabolism.

Methods

Patients: Fasting plasma samples were obtained from two individuals with TD and from patients with LCAT deficiency, analphalipoproteinemia, Hepatic lipase deficiency (HL), vasculitis and hypoalphalipoprteinemia. Their clinical and laboratory findings have been previously reported. Assay: CETP activity was measured using commercial CETP Fluorescence kit (Roar Biomedical Inc., New York, NY). The kit includes donor (without apoA-I) and acceptor lipoprotein particles. Incubation of donor and acceptor with a CETP source results in the CETP mediated transfer of fluorescent neutral lipid from donor to acceptor, rate of which is determined by the increase in fluorescence intensity as the fluorescent neutral lipid is removed from the donor to the acceptor. The amount of fluorescent substrate transferred was expressed as pmoles of fluorescent substrate transferred within 3 hours. CETP concentration was measured by ELISA using specific rabbit antibody against human CETP.

Results

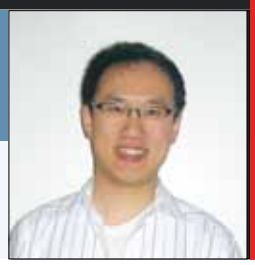
While CETP mass correlated with serum HDL-C levels, the activity decreased in patients with LCAT deficiency, FED, HL deficiency and vascultis, but increased in TD. We speculate that the composition of HDL particles in these disorders differs and results in the different CE transfer rates.

Conclusion

CETP mass correlates with the HDL-C concentration but the activity differs widely among the patients with rare disorders of HDL metabolism, probably due to the changes in HDL quality.

Vincent Fung[1], Morris Pudek[1,2], Frances Rosenberg[1,3], Daniel Holmes[1,3]

[1]University of British Columbia Dept of Pathology and Laboratory Medicine, [2]Dept of Pathology and Laboratory Medicine, Vancouver General Hospital, [3]Dept of Pathology and Laboratory Medicine, St. Paul's Hospital



Vincent Fung

Abstract # 5

Re-evaluation of the correction factor for ethanol in the calculation of the osmolal gap

Backround/Objectives

It is well-known that ethanol (EtOH) demonstrates non-ideal solute behaviour in plasma. This is reflected by its larger than expected contribution to the plasma osmolality. Published multaplicative correction factors for the EtOH contribution range from 1.20 to 1.25. The objective of this study is to determine an optimal correction factor specific to the instrumentation at Vancouver General (VGH) and St. Paul's (SPH) Hospitals.

Methods

Laboratory data from patients presenting to the two respective emergency Dept between August 01, 2007 and November 30, 2008 were extracted from the Sunset database. Plasma sodium, urea, glucose, and EtOH were measured using the two high-volume chemistry analyzers employed at the sites: the Siemens (previously Dade) RXL (VGH) and the Siemens (previously Bayer) Advia 1650 (SPH). Plasma osmolality was measured by freezing-point depression and calculated (excluding the EtOH contribution) using the following standard formula (in SI units):

$$2\,[Na] + [Urea] + [Glucose]$$

Patients without EtOH data or who had undetectable EtOH were excluded as were patients with methanol or ethylene glycol present. Standard regression statistics were employed.

Results

Twelve hundred and fifty-three patient samples (n=823 from SPH and n=430 from VGH) were included. Empirical correction factors m, satisfying

$$\text{Osmol gap (mmol/kg)} = m[EtOH]\ \text{(mmol/L)}$$

were found to be 1.21, 1.17 and 1.18 for VGH, SPH and both combined.

Conclusion

Optimal correction factors for the EtOH contribution to the plasma osmolality appear to be method specific and should be validated at each individual centre and re-evaluated before changes in EtOH methodology.

Abstract # 6

Tony Ng

Tony Ng[1, 2,] Gabriel Leprivier[1], Matthew Robertson[1], Elai Davicioni[1], Timothy Triche[3], Poul Sorensen[1,2]

[1]Dept of Molecular Oncology, British Columbia Cancer Research Centre, Vancouver, BC,
[2]Dept of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC,
[3]Dept of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, Los Angeles

The anoikis stress response induces the AMPK energy-sensing pathway to promote survival in transformed cells

Backround/Objectives

Anoikis is the physiologic response of non-hematopoetic cells to detachment from the extracellular matrix (ECM), in which detached cells undergo rapid apoptosis. Conversely, suppression of anoikis is a hallmark of cancer cells that allows them to survive under anchorage independent conditions such as within the lymphovascular spaces following local invasion from the primary tumor. This process is likely an early and critical step in metastasis. Ewing family tumors (EFTs) are a type of childhood sarcoma with a high propensity for metastasis. Mechanisms mediating suppression of anoikis in EFT, and in sarcomas in general, remain poorly understood.

Methods

To investigate the pathways involved in suppression of anoikis in sarcomas, we performed whole exon gene expression profiling in highly metastatic EFT cell lines in the early stages of anoikis stress. TC32 and TC71 EFT cell lines were forced to grow in suspension by culturing them on non-adherent plates. RNA was isolated at 1, 6 and 24 hours post-transfer and expression profiles were compared to those of control standard monolayer cultures of the same lines using Affymetrix Exon 1.0ST arrays. Biochemical and functional investigations were performed to characterize hits identified from the expression profile analysis.

Results

Expression profiling of EFT cells showed broad and rapid changes in gene expression even at 1 hour following detachment. When compared to reference stress responses we previously obtained from control HEK293 cells subjected to hypoxia, nutrient starvation, endoplasmic reticulum stress or irradiation, there was considerable overlap with each of these four prototypical stress responses as determined by Gene Set Enrichment Analysis (GSEA, $p<0.05$). Amongst the most prominent changes, however, was down-regulation of a distinct group of genes that point to induction of the AMP-activated protein kinase (AMPK) energy-sensing pathway in detached cells (e.g. Prc, Srepb1c, Torc2, Pepck, Pten), reflective of the changes seen during nutrient starvation. We show that multiple downstream branches of the AMPK pathway are indeed activated in non-adherent EFT cells (e.g. p-ACC1, p-p53, p-eEF2, p-AMPK). This was associated with suppressed ATP levels in non-adherent conditions, suggesting that such cells are indeed under bioenergetic stress leading to AMPK pathway activation. Moreover, using AMPK-/- versus wildtype mouse embryonic fibroblasts (MEFs) transformed with activated K-Ras or ETV6-NTRK3 (EN) oncoproteins, we show that survival of transformed MEFs in suspension is lost in the absence of AMPK. Interestingly, reduced mTOR pathway activity was also seen in association with AMPK activation during anoikis stress only in the surviving transformed cells.

Conclusion

Our data indicate that among the multiple stress response pathways that are induced under anchorage independent conditions, activation of the AMPK pathway may represent a critical mechanism for suppression of anoikis in transformed cells. Further investigations into the role of AMPK-mediated mTOR pathway inactivation in promoting anoikis suppression are being performed.

R Ali[1], A Zayed[2], A Dastranj[4], A Elomami[1], E Mehl[3], J Senz[2], CH Lee[1], CB Gilks[1,3], D Huntsman[2,3]

[1]Pathology and Laboratory Medicine, Vancouver General Hospital, Vancouver BC, Canada, [2]Centre for Translational and Applied Genomics (CTAG), BC Cancer Agency, Vancouver BC, Canada, [3]Genetic Pathology Evaluation Centre (GPEC), Vancouver BC, Canada, [4]Pathology Deptt, Medicine Faculty, Tabriz University of Medical Science, Tabriz, Iran

Rola Ali

Abstract # 7

Non-overlapping molecular abnormalities in endometrial stromal neoplasms compared to uterine tumors resembling ovarian sex cord tumor (UTROSCT)

Backround/Objectives

Endometrial stromal sarcoma (ESS) account for 20% of all uterine sarcomas. The translocation t(7;17) (p15;q21) is the most common translocation in ESS and has been demonstrated in about 50% of cases studied to date but not in other uterine sarcomas. This translocation results in JAZF1-JJAZ1 gene fusion. More recently, other translocations have been reported in ESS including JAZF1-PHF1 and EPC1-PHF1. The JAZF1, JJAZ1, and PHF1 genes all encode zinc finger domains often found in DNA-binding proteins. UTROSCT, on the other hand, has not been linked to any specific genetic abnormality.

Objectives

1. To determine the frequency of JAZF1-JJAZ1, JAZF1-PHF1 and EPC1-PHF1 in ESS and morphologically similar tumors by FISH.
2. To determine whether a mutation in the gene FOXL2, which has been found in ovarian granulosa cell tumors, is present in ESS and UTROSCT's.

Methods

A total of 59 formalin-fixed paraffin-embedded tissue samples were studied by FISH. These included 2 endometrial stromal nodules (ESN), 23 ESS, 6 undifferentiated sarcomas, 16 adenosarcomas, 2 MMMTs, 2 UTROSCTs, 1 leiomyosarcoma, and 7 polypoid endometriosis. For FISH, both break-apart and fusion probes were used to study different translocations involving JAZF1, JJAZ1, EPC1, PHF1. TaqMan PCR was performed on these cases and also on 5 additional cases of UTROSCT to test for FOXL2 mutation.

Results

21 out of 23 cases of ESS were assessable by FISH. 7/21 ESS had the JAZF1/JJAZ1 fusion, 3/21 ESS had the JAZF1/PHF1 fusion, and 1/21 ESS had the EPC1/PHF1 fusion. Also, 1 ESS showed only JAZF1 rearrangement, 1 only PHF1 rearrangement, and 1 was equivocal for PHF1 rearrangement, with no fusion partner identified in these cases. No rearrangements were identified in the 7 other ESS or any of the other non-ESS cases including the 2 ESNs. A mutation (402C>G) in the FOXL2 gene was detected in 2/7 UTROSCTs, but not in any other cases.

Conclusion

FISH assays for JAZF1, JJAZ1, EPC1, and PHF1 are specific for ESS but the abnormalities are not universally present. In contrast, UTROSCT seems to be related to ovarian granulosa cell tumors, sharing a mutation of the gene FOXL2.

Abstract # 8

Terry Jefferson

J. Terry[1], A. DeLuca[1], S. Leung[1], G. Peacock[1], Y. Wang[2], W. M. Elliott[3], D. Huntsman[1]

[1]Centre for Translational and Applied Genomics (CTAG) and [2]Dept of Cancer Endocrinology, BC Cancer Agency, Vancouver, BC, Canada, [3]UBC-James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, Vancouver, BC, Canada

NTRK2 expression predicts improved survival in squamous cell carcinoma of lung

Backround/Objectives

The neurotrophic tyrosine kinase receptors NTRK1 (TRKA) and NTRK2 (TRKB) are oncoproteins belonging to a family of nerve growth factor receptors that normally regulate nervous system development. NTRK1 and 2 abnormalities have been reported in neoplasms of lung. In neuroblastoma, NTRK1 expression portends a better patient outcome; however, the significance of NTRK1 and 2 expression in lung cancer is unclear. This study investigates the relationship between immunohistochemical expression of NTRK1 and 2 in lung cancer and patient outcome.

Methods

Normal control tissues and 686 individual lung cancer cases (adenocarcinoma, adenosquamous carcinoma, bronchioloalveolar carcinoma, carcinoid tumor, large cell carcinoma, large cell neuroendocrine tumor, pleomorhic carcinoma and small cell carcinoma) with clinical outcome data in tissue microarray format are immunohistochemically stained for NTRK1 and NTRK2 using commercially available antibodies, automated staining and standard protocols. Positive staining is defined as any amount of membranous staining within tumor tissue in at least one tumor core. Statistical analyses are performed on SPSS v16.0.

Results

NTRK1 and NTRK2 are highly specific markers of squamous carcinoma compared to other lung carcinoma subtypes (NTRK1: sensitivity = 76%, specificity = 97%; NTRK2: sensitivity = 50%, specificity = 96%). Both NTRK1 and NTRK2 show strong correlation to squamous carcinoma of lung (NTRK1: 204/268 positive, Kendall tau-b = 0.729 $p << 0.05$; NTRK2: 135/268 positive, Kendall tau-b = 0.526 $p << 0.05$). Positive staining is less frequent in adenocarcinomas (NTRK1: 11/236, NTRK2: 11/236), adenosquamous carcinomas (1: 2/3, 2: 1/3), bronchioloalveolar carcinomas (1: 0/8, 2: 0/8), carcinoid tumors (1: 1/92, 2: 1/92), large cell carcinomas (1: 6/55, 2: 4/55), large cell neuroendocrine tumors (1: 0/6, 2: 2/6), pleomorphic carcinomas (1: 0/5, 2: 0/5) and small cell carcinomas (1: 0/12, 2: 0/12). There is significant correlation between positive NTRK2 staining and improved disease specific survival (Log rank test: chi2 = 13.4, p = 2.5x10-4) and improved overall survival (chi2= 8.9, p = 2.8x10-3) in squamous carcinoma. NTRK1 staining has no prognostic significance. Positive NTRK2 staining is not frequent enough in other lung cancer subtypes to achieve statistically reliable correlations with patient outcome. NTRK1 staining is present in normal squamous epithelium from skin and tongue (3/3) but not normal lung (0/1) while NTRK2 staining is absent in lung (0/1) or squamous epithelium (0/3) and appears to be cancer specific.

Conclusion

Positive NTRK2 staining predicts significantly improved disease specific and overall survival in patients with squamous carcinoma of lung and may warrant consideration as a clinical prognostic marker. Both NTRK1 and 2 are expressed in squamous lung carcinoma but NTRK2 appears restricted to neoplastic squamous epithelium and may identify a subset of lung cancer with unique pathogenesis amenable to therapies targeting the NTRK2 signaling pathway.

David F Schaeffer[1], Daniel R Owen[1], Howard J Lim[2], Erika Mehl[1], Andrew K Buczkowski[3], Stephen W Chung[3], Charles H Scudamore[3], David G Huntsman[1], Sylvia SW Ng[2], David A Owen[1]

[1]Dept of Pathology and Laboratory Medicine, [2]Dept of SurgerySurgery, The University of British Columbia and [3]BC Cancer Agency, Vancouver, Canada

David F. Schaeffer

Abstract # 9

Insulin-like growth factor-2 mRNA binding protein 3 (IGF2BP3) expression may be a marker for a unfavorable prognosis in pancreatic ductal adenocarcinoma

Backround/Objectives

Pancreatic adenocarcinoma is a lethal disease with a 5-year survival rate of 4% and typically presents in advanced stages. In this setting therapeutic markers identifying aggressive tumors, could aid in management decisions. Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3, also known as IMP3 or KOC) is an oncofetal RNA-binding protein regulating targets such as insulin-like growth factor 2 (IGF2) and ACTB (beta-actin) and has previously been demonstrated to be expressed in pancreatic adenocarcinoma.

Methods

The expression of IGF2BP3 was evaluated by immunohistochemistry on a tissue microarray of 128 pancreatic adenocarcinoma with tumor grade 1, 2 and 3, according to WHO criteria. Non-neoplastic pancreatic parenchyma served as control tissue. Slides were reviewed by two pathologists, blinded to clinical outcome, and scored. Cut-off point for positive cases was any convincing cytoplasmic expression in more than 5% of tumor cells. Univariate disease specific survival analysis was performed by the generation of Kaplan- Meier curves and differences assessed with the log-rank statistic.

Results

IGF2BP3 was found to be selectively overexpressed in pancreatic ductal adenocarcinoma tissues but not in benign pancreatic tissues, as previously reported. 9 (40%) patient samples of tumor grade 1 (n=24) and 27 (44%) of tumor grade 2 did express IGF2BP3. The highest rate of expression was seen in poorly differentiated specimen (Grade 3, n=42) with 26 (63%) positive samples. Overall survival was found to be significantly shorter in patients with IGF2BP3 expressing tumors (p=0.001; RR 2.3, 95% 1.2-4.8).

Conclusion

Although IGF2BP3 is expressed in a variety of malignant neoplasm (i.e. pulmonary SCC, ovarian CCC and RCC) prognostic value has only be demonstrated in RCC. Our data suggest, that IGF2BP3 denotes a subset of pancreatic adenocarcinomas with an extremely poor outcome and provides a rationale for developing therapies to target the IGF pathway in this cancer.

Abstract # 10

Karen Ung

Karen Ung, Torsten Nielsen, Stephen Chia, Vivian Bramwell, Lois Shepherd, Dongsheng Tu, Kathy Pritchard

The prognostic and predictive significance of biological classification of primary breast cancer with tissue microarray from NCIC CTG MA12 trial

Backround/Objectives

Breast cancer is a heterogenous disease that can be classified into at least four major biologically distinct subtypes by gene expression profiling (luminal A, luminal B, human epidermal growth factor receptor 2 overexpressing, basal-like), and prognostic implications of these molecular subtypes have been described. Using tumor tissues and data from a large prospective randomized trial of premenopausal patients with early stage breast cancers (NCIC CTG MA12), our objective is to assess breast cancer specific survival as stratified by tamoxifen treatment using previously established immunohistochemical surrogate panels for breast cancer intrinsic subtype. Specifically, the goals of this study are:

1. To assess the prognostic significance of the 6 antibody surrogate panel.

2. To assess the predictive significance of immunohistochemical based biological classification, luminal A versus luminal B, for benefit for tamoxifen.

3. To assess the predictive significance of quantitative estrogen receptor expression.

4. To compare the protein based immunohistochemical panel of biomarkers utilized for biological classification to that of gene expression profiling with qRT-PCR..

Methods

846 tissue blocks representing 520 of the 672 cases in the NCIC CTG MA12 study were collected, of which 492 cases have invasive breast carcinoma used to build tissue microarray. ER, PR, HER2, Ki67, EGFR, and CK 5/6 immunostains were used to define the four major breast cancer intrinsic subtypes (luminal A, luminal B, HER2 overexpressing, basal-like). The prognostic and predictive significance of the immunohistochemical based biological classification were assessed using data from MA12 trial (median followup 9.7 years) against the study outcomes of overall- and disease free survival. ER expression was quantified using the Ariol system, and parallel tissue cores were collected for qRT-PCR using the PAM50 method.

Results

The distribution of breast cancer biologic subtypes by immunohistochemistry is as follows: 39% luminal A, 39% luminal B, 21% basal-like, 7% HER2, and 4% unassignable. These subtypes have similar prognostic significance as compared to those from large regional population-based cohort study.Specifically, the 5 year overall survival among different subtypes show trend toward statistical significance, with the basal-like doing worse than luminal A. Among the luminal subtypes, there is a trend for luminal B to have greater benefit of tamoxifen as compared to luminal A. Quantitative ER and RT-PCR datasets are complete and submitted to the NCIC-CTG central statistical office for correlation to patient outcome.

Anna F. Lee[1], Cheng-Han Lee[1], Malcom Hayes[2], Torsten O. Nielsen[1,3]

[1]Dept of Anatomical Pathology, Vancouver General Hospital, Vancouver, Canada; [2]Dept of Pathology, BC Cancer Agency, Vancouver, Canada; [3]Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, Canada

Anna Lee

Abstract # 11

FLI-1 is a specific marker of Ewing sarcoma in non-hematolymphoid small round blue cell tumors of bone

Backround/Objectives

Ewing sarcoma/primitive neuroectodermal tumour (PNET) and small cell osteosarcoma (OSA) are two types of bone tumors with small round blue cell morphology, which have vastly different prognoses and treatment modalities. Both tumors can show similar morphology on routine sections. The two tumors are thus often considered together in the differential diagnosis, along with some hematolymphoid neoplasms, mesenchymal chondrosarcoma, rhabdomyosarcoma, neuroblastoma, and other small blue cell tumors. While the presence of osteoblastic and/or chondroblastic elements are diagnostic of OSA regardless of the size of the blue cell component, it is not uncommon for the biopsy to consist of blue cells only, in which case the diagnosis is highly reliant on immunohistochemical and molecular studies. FLI-1, a protein expressed in normal blood vessels as well as Ewing sarcoma/PNET, is expressed in some B-cell lymphomas, but negative in mesenchymal chondrosarcoma, rhabdomyosarcoma, and neuroblastoma. However, its expression in small cell OSA is still uncharacterized. We therefore investigated whether FLI-1 was expressed in small cell OSA.

Methods

All formalin-fixed paraffin embedded blocks of biopsy or resection specimens of small cell OSA, from 1994-2008, were collected from VGH archives. Slides were re-reviewed by CHL and AL to confirm the diagnosis. Blocks with demonstrable small cell OSA morphology were stained for H&E and immunostained for FLI-1. FLI-1 staining intensity and pattern in each case was scored, and the percentage of cases with positive FLI-1 immunoreactivity were calculated. Internal positive control consisted of positive staining of normal blood vessels. As well, some cases of Ewing sarcoma/PNET with molecularly confirmed EWS-FLI-1 gene fusion were stained for FLI-1 along with the osteosarcoma cases.

Results

Of a total of 9 tumors with small cell OSA morphology examined to date, none (0%) of the tumour cells stained for FLI-1 although background normal blood vessels were positive. In contrast, 7/7 (100%) of the Ewing sarcoma/PNET cases showed moderate to strong FLI-1 staining intensity in the tumours as well as the background normal blood vessels.

Conclusion

Together with previous studies demonstrating FLI-1 negativity in mesenchymal chondrosarcoma, rhabdomyosarcoma, and neuroblastoma, our preliminary data, which demonstrate a complete lack of FLI-1 reactivity in small cell OSA, suggest that FLI-1 is a specific marker of Ewing sarcoma/PNET in non-hematolymphoid small round blue cell tumors of bone.

Abstract # 12

Fang-I Lu

Fang-I Lu[1], Douglas Webber[1,2], De Wet van Niekerk[3], Susan Tha[1,2], David Owen[1,2], Malcolm Hayes[4]

[1]Dept of Pathology and Laboratory Medicine, Vancouver General Hospital, [2]Dept of Pathology, University of British Columbia Hospital, [3]University of British Columbia, Dept of Biophysics, [4]British Columbia Cancer Agency, Dept of Pathology

Longitudinal study of sessile serrated adenoma of the colorectum

Backround/Objectives

Sessile serrated adenoma (SSA) is a distinct entity differentiated from the common hyperplastic polyp (HP) of the colorectum by morphology, immunophenotype, mucin phenotype and genetic characteristics. SSA has been found to be significantly more often microsatellite-unstable, and can be associated with synchronous or metachronous colorectal carcinoma which may be microsatellite unstable. Due to these associations, SSA is thought to be a possible precursor to colorectal carcinoma via the serrated pathway, and it is recommended that SSA be treated similarly to conventional adenomatous polyp (AP). Nevertheless, to our knowledge, no true longitudinal study concerning SSA has been published to date. The primary aim of this project is to retrospectively study the clinical outcome of SSA, and compare it with that of the HP and AP.

Methods

Selection of study group: A total of 1402 cases of colorectal polyp originally diagnosed as HP were selected from the University of British Columbia Hospital archive. These had been endoscopically resected or biopsied between 1980 and 2001 by different gastroenterologists. H&E stained sections were reviewed by a pathology resident (F.L.) or research assistant (D.V.N.), and any polyps felt to be diagnostic or suspicious of SSA or other non-hyperplastic polyps were additionally reviewed by a pathologist (D.W.). Polyps diagnosed as SSA were then shown to a panel of two additional pathologists (S.T., D.O.). Only cases diagnosed as SSA by all three pathologists (D.W., S.T., D.O.) were used in this study. Selection of control groups: Controls were chosen on a per-case basis. For each SSA, control AP and HP were chosen. Subjects for control HP needed to be free of previous or synchronous diagnosis of higher risk lesion(s) on initial presentation. Subjects for control cases were sex-matched and age-matched within 10 years compared to the subjects with SSA. H&E stained sections for the control cases of hyperplastic polyp were also reviewed. Clinical Follow-up: Clinical follow-up was obtained for each SSA and its control AP and HP. Follow-up was available through a variety of sources: the internal surgical pathology database, the patient charts at the University of British Columbia Hospital, and the BC Cancer Registry.

Results

This is an ongoing research project with data still being gathered/analyzed.

K. Tan[1], M. Anderson[2], M. Krajden[3], M. Petric[3], A. Mak[3], M. Naus[2]

[1]Dept of Pathology and Laboratory Medicine, UBC, Vancouver, Canada, [2]Epidemiology Services, British Columbia Centre for Disease Control, Vancouver, Canada, [3]Laboratory Services, British Columbia Centre for Disease Control, Vancouver, Canada

Kennard Tan

Mumps virus detection by RT-PCR and culture during an outbreak in a predominantly unvaccinated population

Backround/Objectives

Control measures for mumps during an outbreak consist of immunization of susceptible and isolation of infected persons. Recently, shortening the isolation period from 9-days to 5-days after the onset of parotitis has been recommended by the CDC. This was based on historical studies and one small 2008 study on a highly vaccinated population exposed to mumps. In British Columbia, mumps circulated in a predominantly unvaccinated population from February to October 2008. We analyzed the duration of mumps viral detection after symptom onset.

Methods

Demographic, clinical, and laboratory data were collected in a standardized manner during the course of the outbreak. Serology, buccal swabs and/or urine samples were tested at the BC Centre for Disease Control. Specimens had been collected at varying periods after onset of parotitis. Specimens were tested for mumps virus by isolation in cell culture and RT-PCR. Viral detection in buccal specimens was analyzed relative to the number of days after parotitis onset.

Results

180 documented cases of mumps were identified in this outbreak, and clinical data was available for 91% of cases. Of these 47% did not recall a history of immunization. 62 buccal specimens were available for assessment. Mumps virus was detected by both PCR and culture until at nine days post-parotitis onset. Mumps PCR had a higher detection rate than culture.

Conclusion

The rate of viral detection was highest in specimens collected immediately after the onset of parotitis, and decreases soon after. However, virus could be detected in a subset up to day-9, suggesting that a 5-day isolation period post-parotitis may not be sufficient in controlling infection. Our observations are based on single specimen submissions with clinical data collected retrospectively; a prospective study with serial specimen collection after presentation would be ideal. Further investigations are warranted to validate patient isolation guidelines.

Abstract # 14

Aleksandra Stefanovic

A Stefanovic[1], V. Montoya[2], A. Chan[3], F. Brinkman[4], S. Jones[5], L. Hoang[3], P. Tang[3]

[1]Division of Medical Microbiology, Dept of Pathology and Laboratory Sciences, UBC, [2]Biological Sciences, UBC, [3]Dept of Pathology and Laboratory Medicine, UBC and Laboratory Services, BC Centre for Disease Control, PHSA, [4]SFU, 5BC Genome Sciences Centre

Identification of a novel genomic island in invasive streptococcus pneumoniae serotype 5 outbreak strain

Backround/Objectives

Streptococcus pneumoniae serotype 5 was responsible for a large community outbreak in Vancouver, BC in the winter of 2006-2007. Infections with this strain caused highly invasive pneumococcal disease (IPD) and were associated with significant morbidity. Our aim is to determine the genetic factors conferring increased virulence in this particular strain of S. pneumoniae serotype 5.

Methods

During the outbreak, 205 S. pneumoniae samples were serotyped. Of these, 184 (90%) belonged to serotype 5 and the rest belonged to other serotypes. One representative isolate was fully sequenced using an Illumina Genome Sequencer at the BC Genome Sciences Centre. The genome sequence was compared to available Streptococcus sequences in National Center for Biotechnology Information (NCBI) Genbank and a unique genomic island was found. We designed Polymerase Chain Reaction (PCR) assays to screen for this island in the other isolates of S. pneumoniae before, during and after the outbreak. We also looked for the presence of the pathogenicity island among invasive versus non-invasive isolates of S. pneumoniae serotype 5.

Results

The nucleotide sequence of the genomic island was most closely related to sequences from Streptococcus suis indicating a possible recent horizontal gene transfer event. Characterization of the genomic island showed that it contains genes encoding proteins of the Entner-Doudoroff pathway. This pathway is involved in complex carbohydrate metabolism. Recent evidence suggests that complex carbohydrate utilization has a role in pathogenesis of streptococci. The enzymes involved in glycolysis appear to have not only a role in utilizing a wider range of energy sources but also in epithelial adherence and degradation of host polymers such as those found in mucus.

Conclusion

A novel genomic island containing genes derived from S. suis has been discovered in a recent outbreak strain of S. pneumoniae. Its role in utilizing complex sugars may confer some survival advantage to these pneumococcal strains by access to a wider range of energy sources. We will be conducting further studies to ascertain if these enzymes contribute to the breakdown of host glycoproteins, aid in adherence and have a role in pathogenicity.

Miguel Imperial[1], Lorraine Vowles[2], John Galbraith[1,2], Pamela Kibsey[1,2]

[1]Dept of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada, [2]Dept of Laboratory Medicine, Vancouver Island Health Authority, Victoria, Canada

Miquel Imperial

Performance of an algorithm using techLab C. DIFF CHEK and techlab C. DIFFICILE TOX A/B II as compared to BD cdiff GeneOhm PCR for the detection of clostridium difficile in stool samples

Backround/Objectives

Clostridium difficile is the leading cause of nosocomially acquired diarrhea. New rapid tests have been developed, with PCR emerging as a new gold standard. We compare the performance of an algorithm that uses glutamate dehydrogenase enzyme immunoassay, C. DIFF CHECK (GDH), as a screen followed by a toxin A/B enzyme immunoassay, C.DIFF TOX A/B (TOX A/B), to the BD GeneOhm Cdiff tcdB gene PCR.

Methods

Stool samples meeting criteria were tested for C. difficile in parallel using both PCR and GDH. GDH positive samples were tested by TOX A/B. TOX A/B negative samples were tested by cytotoxin cell culture. Results were compared to PCR.

Results

97 samples were tested. With PCR as the reference, 12 (12%) were positive. 76 samples were PCR and GDH negative, with no samples GDH negative but PCR positive. 9 samples were GDH positive but PCR negative. The sensitivity and specificity of GDH alone was 100% (76/76) and 89% (76/85), for a NPV and PPV of 100% (76/76) and 57% (9/21). The 21 GDH positive results were tested by TOX A/B, which detected 5 of 12 that were PCR positive. There were no TOX A/B positive, PCR negative samples, resulting in a sensitivity and specificity of 42% (5/12) and 100% (9/9) and a NPV and PPV of 56% (9/16) and 100% (5/5). The 7 TOX A/B negative and the 9 GDH positive, PCR negative samples were tested by cytotoxin cell culture. Of these 16, cytotoxin cell culture compared to PCR had a sensitivity of 57% (4/7), a specificity of 89% (8/9), a PPV of 80% (4/5) and a NPV of 75% (9/12).

Conclusion

The performance of GDH assay as a screening test is excellent, with a NPV of 100%. Combined with the 100% specific TOX A/B assay, an algorithm using these tests allows for fast reporting of GDH negative or TOX A/B positive samples, which are the majority. PCR, as the emerging reference standard, can be used to rapidly assay GDH positive, TOX A/B negative samples.

Abstract # 16

Henry Stringer

Henry Stringer, John Maguire, Hélène CF Côté

Dept of Pathology & Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Mitochondrial DNA (mtDNA) depletion and deletion in statin-induced myopathy

Backround/Objectives

Mitochondrial dysfunction occurs in a wide variety of diseases and has been implicated with some drug toxicities. Statins are widely used to treat hyperlipidemia but cause statin-induced myopathy or SIM (a potentially fatal complication) in ~5-15% of patients. The contribution of mitochondrial dysfunction to the development and exacerbation of SIM may be important. In order to further analyze this, we developed a scale for quantifying mtDNA deletion patterns to facilitate the analysis of mtDNA deletions in clinical samples of muscle biopsies from patients with SIM, in order to evaluate whether changes in mtDNA quantity or quality (deletions) could be detected, and provide further insight into the etiology and severity of mitochondrial myotoxicity in SIM.

Methods

Muscle biopsies from patients diagnosed with SIM (n=24) and comparators showing no pathologic findings (n=25) were retrospectively identified from stored cryoprotected clinical muscle biopsies. The degree of pathology within each biopsy was scored from 0 to 3. PCR quantification of mtDNA and nuclear DNA (nDNA) was used to measure mtDNA/nDNA ratio. Long-template PCR was used to amplify the mitochondrial genome in two fragments. MtDNA deletion patterns were visualized via agarose gel electrophoresis, digitized, and scored using an in-house scale from 0 to 200. Differences in mtDNA content and deletion score were compared between SIM and comparator groups by t-test or Mann-Whitney test.

Results

SIM sample mtDNA content (mean ± SD, 2035 ± 1146) was 35% lower than that of the comparators (3127 ± 1159) (p=0.008). MtDNA deletions were mostly confined to one of the PCR fragments. Although mtDNA deletions were seen more frequently in SIM samples (21.2 ± 19.2) than in comparators (19.9 ± 28.8), this difference did not reach statistical significance. No correlation was seen between age and mtDNA content as well as between age and mtDNA deletions. There was an inverse correlation between mtDNA content and pathology score (p=0.001, r=-0.443).

Conclusion

We found decreased muscle mtDNA quantity in association with SIM, and this is consistent with previous work. How this relates to the pathogenesis of SIM remains unclear. As the mtDNA deletion score was not associated with SIM, quantitative rather than qualitative mtDNA alterations are more likely implicated. We recommend that mtDNA quantity should be further investigated as a potential marker of drug myotoxicity.

Tyler B.M. Hickey[1], Hermann J. Ziltener[1,4], David P. Speert[1, 2, 3] and Richard W. Stokes[1, 2, 3]

Depts of Pathology & Laboratory Medicine1, Paediatrics 2, Division of Infectious and Immunological Diseases (British Columbia's Children's Hospital)3, Biomedical Research Centre4, at the University of British Columbia, Vancouver, British Columbia, Canada

Tyler B.M. Hickey

Identification of Cpn60.2 as a surface ligand of mycobacterium tuberculosis that facilitates bacterial association with macrophages via CD43

Backround/Objectives

Mycobacterium tuberculosis bacilli initially contact host cells with elements of their outer cell wall, or capsule. We have previously shown that proteins from the surface of *M. tuberculosis* competitively inhibit the non-opsonic binding of whole *M. tuberculosis* bacilli to macrophages in a dose-dependent manner. CD43 is a large sialylated glycoprotein that is found on the surface of macrophages and has been shown in previous studies to be necessary for efficient macrophage binding and immunological responsiveness to *M. tuberculosis.* However, a mycobacterial ligand for CD43 never been described. The hypothesis of our research was that an as yet unidentified mycobacterial ligand(s) exists that mediates *M. tuberculosis* binding to CD43, and therefore, to the M *phi*.

Methods

Employing the use of a CD43-Fc chimeric glycoprotein, we designed an affinity chromatography system to immobilize CD43-Fc and identify CD43-specific mycobacterial ligands using capsular material from *M. tuberculosis.* Identified ligands from this procedure were subsequently tested for their capacity to act as adhesins for macrophage binding using recombinant protein competitive inhibition and antibody-mediated epitope masking strategies. Additionally, for identified ligands that bound to CD43 in vitro AND showed adhesin function upon macrophages, we evaluated the capacity of these identified M. tuberculosis adhesins to bind macrophage-presented CD43 by comparing binding the inhibition strategies with both CD43+/+ and CD43-/- macrophages.

Results

The *M. tuberculosis* capsule contains the molecular chaperones Cpn60.2 (Hsp65, GroEL) and DnaK (Hsp70) and each of these proteins binds to immobilized CD43-Fc in an affinity chromatography format. However, when each of these proteins was tested for macrophage adhesin function, Cpn60.2, but not DnaK, mediated M. tuberculosis binding to the macrophage surface. Furthermore, Cpn60.2 appears to require macrophage CD43 to facilitate bacterial binding, as the binding inhibition methods specific to Cpn60.2 were not functional when using CD43-/- macrophages. Lastly, the bacterial homologue GroEL from E. coli also demonstrated CD43-specific adhesin characteristics, but Hsp60 homologues from mouse and human sources did not show adhesin function.

Conclusion

The *M. tuberculosis* molecular chaperones Cpn60.2 and DnaK are both found in the capsule and on the surface of the bacteria. Both Cpn60.2 and DnaK also bind to CD43-Fc in an affinity chromatography format, but only Cpn60.2 employs this function with regards to facilitating bacterial association with macrophages. Furthermore, other bacterial molecular chaperones appear to have the capacity to bind macrophage CD43, but this function is not maintained by mammalian homologues. Thus, our findings add to a growing field of research that ascribes non-traditional localization and functions to molecular chaperones that were previously only associated with protein folding activities within the cytosol.

Abstract # 18

Jerry Wong

Jerry Wong, Jingchun Zhang, Gloria Fong, Bruce McManus, Honglin Luo

Dept of Pathology and Laboratory Medicine, The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, University of British Columbia, Providence Heart + Lung Institute, St. Paul's Hospital

The role of serum response factor in coxsackieviral cardiomyopathy

Backround/Objectives

Coxsackievirus B3 (CVB3) is the most common pathogen associated with viral myocarditis and its sequelae, dilated cardiomyopathy (DCM). CVB3 encodes two viral proteases (2Apro and 3Cpro) that cleave multiple host proteins essential for maintenance of cellular architecture, protein translation, transcription, and cell-signaling. Serum response factor (SRF) is a cardiac-enriched transcription factor associated with the regulation of contractile genes and miRNAs that silence the expression of cardiac regulatory factors. Disruption of SRF in the heart results in down-regulation of cardiac contractile gene expression and development of DCM. The goal of our research is to understand the role of SRF in coxsackieviral cardiomyopathy.

Methods

Murine cardiomyocytes and A/J mice were infected by CVB3 for different time courses. Cell lysates or heart extracts were collected, and SRF protein and gene expression was examined by Immunoblotting and real-time qRT-PCR, respectively. To determine whether SRF is cleaved by CVB3 proteases, HeLa cells were transiently transfected with an N-terminus flag-tagged SRF alone followed by CVB3 infection or co-transfected with 2Apro or 3Cpro plasmid. SRF expression was analyzed by Immunoblotting using antibodies against Flag and C-terminus of SRF.

Results

CVB3 infection of mouse cardiomyocytes and hearts resulted in marked decreases in SRF protein expression, but not in mRNA expression. Further investigation by antibody epitope mapping demonstrated that SRF was cleaved into 47kDa and 20kDa fragments after CVB3 infection, as predicted by the NetPicoRNA V1.0 algorithm for potential cleavage sites by 2Apro. We further showed that co-transfection of SRF with 2Apro led to a substantial decrease in protein expression of full-length SRF.

Conclusion

SRF is directly cleaved during coxsackievirus infection, likely by 2Apro. Understanding the functional significance of SRF cleavage will provide valuable insights towards the development of viral cardiomyopathy and further the development of new therapeutic approaches to ameliorate myocardial damage and progression to DCM.

Marissa Jitratkosol[1], Beheroze Sattha[1], Evelyn Mann[2], Izabella Gadawski[1], Deborah Money[2,3], John Forbes[2,3], Helene CF Cote[1,3] & CIHR Team Grant in HIV Therapy and Aging-CARMA

[1]Dept of Pathology and Laboratory Medicine, UBC, [2]Oak Tree Clinic, Children's and Women's Health Centres of BC, Vancouver [3]Women's Health Research Institute, Vancouver

Marissa Jitratkosol

Abstract # 19

Mitochondrial DNA (mtDNA) mutations in infants exposed to HAART *in utero*

Backround/Objectives

To prevent the vertical transmission of HIV during pregnancy, HIV+ women receive highly active antiretroviral therapy (HAART), decreasing the risk of transmission from ~25% to ≤1%. Nucleoside reverse transcriptase inhibitors (NRTIs) e.g. AZT are the cornerstone of HAART, and act by inhibiting the HIV reverse transcriptase. NRTIs can also inhibit human polymerase γ, the only enzyme responsible for mitochondrial DNA (mtDNA) replication. This may lead to an increase in mtDNA point mutations which has been linked to many age-associated diseases. Since NRTIs can cross the placenta and accumulate in amniotic fluid, they may induce mtDNA mutations in developing foetuses. HAART decreases vertical transmission of HIV and its benefits clearly outweigh its risks. However, the full impact of these drugs in uninfected infants, especially in the long-term, is largely unknown and needs to be assessed.

Methods

This is a retro/prospective study, whereby blood samples are collected from newborns and their mothers (~50 HAART exposed and ~40 unexposed control pairs collected). The D-loop region of the mtDNA genome is amplified by PCR, cloned (~100 clones per individual) and sequenced. MtDNA of infant and mother pairs will be compared to assess mtDNA mutation burden.

Results

Preliminary results show that control infants showed significantly lower mutation rates (mutations/10000 bp) (mean ± SD, 3.88 ± 1.00) than their mothers (4.62 ± 1.02) ($p = 0.033$, $N = 23$) however this was not the case for the study group (4.25 ± 1.73, 4.38 ± 1.51 respectively, $N = 30$). Between groups there was no difference amongst infants.

Conclusion

We found that control infants had lower mutation rates than their mothers but there was no significant difference between study infants and their mothers; this is consistent with our hypothesis that treatment with HAART is associated with mtDNA mutations. However, this is preliminary data as we have yet to complete the sequencing of all samples.

Abstract # 20

Cleo Y.F. Lee

Cleo Y. F. Lee[1,4], Paul S. Rennie[1,2,4] and William W. G. Jia[3,4]

Dept of [1]Pathology & Laboratory Medicine, [2]Urologic Sciences, and [3]Surgery, University of British Columbia, Vancouver, Canada; and [4]The Prostate Centre at Vancouver General Hospital, Vancouver, BC, Canada

MicroRNA regulation of oncolytic herpes simplex virus type 1 for selective killing of prostate cancer cells

Backround/Objectives

Advanced, castration-resistant prostate cancer, for which there are few treatment options, remains one of the leading causes of cancer death. MicroRNAs (miRNAs) have provided a new opportunity for more stringent regulation of tumor-specific viral replication. The purpose of this study was to provide a proof of principle that microRNA-regulated oncolytic HSV-1 virus can selectively target cancer cells with reduced toxicity to normal tissues.

Methods

We incorporated multiple copies of miRNA complementary target sequences (for miR-143 or miR-145) into the 3' untranslated region (3'UTR) of a herpes simplex virus-1 (HSV-1) essential viral gene, ICP4, to create CMV-ICP4-143T and CMV-ICP4-145T amplicon viruses and tested their targeting specificity and efficacy both in vitro and in vivo.

Results

While miR-143 and miR-145 are highly expressed in normal tissues, they are significantly downregulated in prostate cancer cells. We further demonstrated that miR-143 and miR-145 inhibited the expression of the ICP4 gene at the translational level by targeting the corresponding 3'UTR in a dose-dependent manner. This enabled selective viral replication in prostate cancer cells. When mice bearing LNCaP human prostate tumors were treated with these miRNA-regulated oncolytic viruses, a >80% reduction in tumor volume was observed, with significantly attenuated virulence to normal tissues in comparison to control amplicon viruses not carrying these 3'UTR sequences.

Conclusion

Our study is the first to show that inclusion of specific miRNA target sequences into the 3'UTR of an essential HSV-1 gene is a viable strategy for restricting viral replication and oncolysis to cancer cells while sparing normal tissues.

Darrell C. Bessette[1,4], Catherine J. Pallen[1,2,3,4]

Depts. of [1]Pathology and Laboratory Medicine, [2]Experimental Medicine, and [3]Pediatrics, [4]Child & Family Research Institute, Vancouver, British Columbia, Canada

Darrell Bessette

The role of PRL-3 in mediating the effects of p53 in prostate and breast cancer cell lines

Backround/Objectives

PRL-3 is a member of a novel subfamily of protein tyrosine phosphatases (PTPs) that possess a C-terminal CAAX box motif involved in prenylation-dependent membrane targeting of these proteins. PRL-3 is consistently upregulated in a panel of liver metastases derived from colon carcinomas while exhibiting low expression in primary tumors and little to no expression in normal colon epithelia. Furthermore, overexpression of PRL-3 promotes cell migration and invasion abilities, and permits formation of metastases in vivo. Recent research has shown PRL-3 to be a p53-regulated gene in mouse embryonic fibroblasts which is upregulated upon DNA damage. This can lead to G1 cell cycle arrest but, interestingly, PRL-3 knockdown by siRNA may also lead to cell cycle arrest, suggesting the levels of PRL-3 may be important in controlling cell cycle. As p53 is a tumour suppressor, and PRL-3 appears to be a metastasis-associated oncogene, this study will attempt to elucidate the interaction between p53 and PRL-3 in human cancer cell lines.

Methods

We will use the prostate cancer lines, LNCaP, DU-145 and PC-3, as well as the breast cancer line MCF-7, to examine how DNA damage and hypoxic-like conditions can upregulate p53 and affect PRL-3 expression. Then, using siRNA knockdown of PRL-3, we will determine how loss of endogenous PRL-3 expression in these cell lines affect p53, as well as cycle cycle, proliferation, migration and invasion.

Results

Initial evidence suggests that inducing DNA damage or hypoxic-like conditions in LNCaP and MCF-7 cells leads to upregulation of p53 protein expression, but interestingly, no change in PRL-3 protein expression (LNCaP) or a substantial decrease in PRL-3 (MCF-7). MCF-7 cell survival after doxorubicin incubation remains unchanged, while LNCaP and DU145 cell survival is greatly reduced. The role of PRL-3 in proliferation, migration and invasion is being investigated.

Conclusion

Determining the molecular action of PRL-3 in regulating cellular activities will provide important insight into its role in metastasis and cancer and, possibly, identify new targets for therapeutic interventions in advanced disease.

Peyman Tavassoli

Peyman Tavassoli[1, 2], Latif Wafa[1], Helen Cheng[1], Rob Snoek[1], Ladan Fazli[1], Martin Gleave[1], Paul S. Rennie[1, 2]

[1]Prostate Centre at Vancouver General Hospital, and the Dept of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada V6H 3Z6 [2]Dept of Pathology & Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

TAF1, from a general transcription factor to modulator of androgen receptor in prostate cancer

Backround/Objectives

Androgen ablation therapies are still the most effective treatment for advanced prostate cancer (PCa), although it eventually progresses to the lethal ablation-resistant disease. At this stage, most tumour cells retain androgen receptor (AR), which keeps transcriptional activity through alternative pathways, such as aberrant expression of AR-coregulators. AR-coregulators are proteins that interact with AR to influence its activity in a tissue specific manner. Hence, our laboratory used the repressed transactivator yeast two-hybrid system to find novel proteins that interact with the N-terminus of AR using a prostate cancer cDNA library and the TATA binding protein associated factor 1 (TAF1) was identified. TAF1 could activate or suppress transciption of several genes through its functional domains, which are capable of phosphorylation, acetylation, ubiquitin activation and conjugation.

Methods and Results

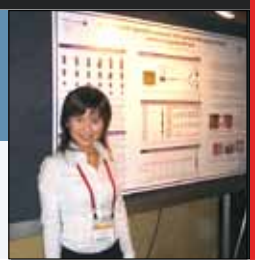
To find out whether TAF1 expression level is changed during PCa progression, human PCa tissue microarray of hormone naive, neoadjuvant hormone-treated (NHT) and ablation-resistant samples were stained for TAF1 protein. The results showed that TAF1 expression significantly increases after 3 months of NHT therapy and with progression to ablation-resistant disease. To confirm AR/TAF1 binding and determine the domains involved, GST-fusion proteins of various AR domains were purified and incubated with radiolabel full-length TAF1 or one of its four truncated TAF1 domains before analysing by SDS-PAGE and autoradiography. Our data indicated that the N-terminus of AR bound to TAF1 most likely through its acetylation and ubiquitin-activating/conjugating (E1/E2) domains. Furthermore using LNCaP cells, an AR-expressing prostate cancer cell line, we showed by co-immunoprecipitation that TAF1 and AR associated in the nucleus. Using ChIP assays with LNCaP cells, we also found that both AR and TAF1 associated together on the proximal promoter of the PSA gene. To investigate whether TAF1 could affect AR transcriptional activity, transactivation assays were performed. Our data showed that TAF1 differentially enhances AR activity through its N-terminal kinase (NTK) and E1/E2 domains. Using in vivo and in vitro ubiquitination assays, we next found that TAF1 is able to ubiquitinate AR in a spectrum from mono- to poly-ubiquitin conjugated AR.

Conclusion

TAF1 is a specific coactivator of AR that binds and differentially enhances AR transcriptional activity most likely through ubiquitination of AR. Accordingly, an increase in TAF1 expression during NHT therapy for advanced PCa, especially with prolonged treatment of over 6 months, could be a compensatory mechanism adapted by cancer cells to overcome lack of circulating androgens.

Ivy F.L. Tsui[1,3], Catherine F. Poh[2,4], Cathie Garnis[3], Miriam P. Rosin[4], Lewei Zhang[2], and Wan L. Lam[1,3]

Depts of [1]Pathology and Laboratory Medicine, and [2]Oral Biological and Medical Sciences, UBC, Vancouver, British Columbia, and Depts of [3]Cancer Genetics and Developmental Biology, and [4]Cancer Control Research, British Columbia Cancer Research Centre, BC



Ivy Tsui

DNA amplification occurs in oral dysplasias and disrupts multiple genes in one signalling network

Backround/Objectives

Studying oral premalignant lesions (OPLs), the precursor of oral squamous cell carcinomas (OSCCs), is important to the identification of early genetic events that may represent key changes in disease initiation and development. Regions of high-level copy number alterations, including DNA amplification and homozygous deletions, may contain oncogenes and tumour suppressors, respectively. We ask if these regions occur in OPLs and which oncogenic pathways are disrupted in OPLs.

Methods

We evaluated gene dosage in 71 OPLs by whole genome tiling-path microarray. Fluorescence in situ hybridization (FISH) assays were performed with probes spanning EGFR and CCND1. Real-time PCR using total RNA from eight OSCCs and nine normal oral mucosal tissue was performed for two identified genes.

Results

We discovered the presence of gene amplification and homozygous deletions in these early stage samples, with 42.6% (20/47) of high-grade dysplasias and 22.2% (2/9) of low-grade lesions that later progressed to cancer exhibiting these features. Genes within ten recurrent amplicons identified in our set of OPLs were evaluated for overexpression in five independent head and neck cancer datasets. Many of the identified gene candidates are shared components of the canonical FGF, ERK/MAPK, and PI3K/AKT signalling pathways ($P < 6.92 \times 10\text{-}3$). Interestingly, many of the OPLs harbour more than one amplicon, suggesting the dependence on multiple oncogenes for cancer development. Furthermore, co-amplification of EGFR and CCND1 from the same pathway was detected within single cells of oral dysplasias by FISH, extending the concept of "oncogene addiction" to the pathway level.

Conclusion

Taken together, our study demonstrates the need for combined targeting of these oncogenic pathways for the effective treatment of oral cancer patients. Furthermore, genetic testing to detect amplification hotspots might have potential for improved personalized therapies for oral cancer patients.

Agatha N. Jassem

Agatha N. Jassem[1], James E. A. Zlosnik[2], Robert K. Ernst[4], Robert E. W. Hancock[2,3], and David P. Speert[1,2,3]

Depts of Pathology and Laboratory Medicine[1], Pediatrics[2], Microbiology and Immunology[3], University of British Columbia[4], Dept of Microbial Pathogenesis, University of Maryland

Outer membrane mediated aminoglycoside resistance in the burkholderia cepacia complex

Backround/Objectives

The *Burkholderia cepacia* complex (Bcc) is a group of closely-related Gram-negative bacterial species that are highly virulent opportunistic pathogens, particularly in individuals with cystic fibrosis (CF) or chronic granulomatous disease. Treatment of Bcc infections is hampered by their intrinsic resistance to many antimicrobials, including polycationic aminoglycosides (AGs). Mechanisms of AG resistance in the Bcc are thought to involve characteristics of the outer membrane (OM) since cationic antimicrobials rely on lipid A binding sites to enter Gram-negative bacteria through lipopolysaccharide-mediated self-promoted uptake. In a survey of Bcc bacteria we observed that, unlike the other species, most environmental and clinical isolates of B. *vietnamiensis* (*Bv*) were intrinsically susceptible to AGs, yet remained highly resistant to other cationic agents (cationic antimicrobial peptides, polymyxin B). Furthermore, some strains acquired AG resistance during chronic infection. These observations challenge the existing dogma of Bcc resistance and suggest that in *Bv* different mechanisms of resistance exist for different classes of cationic agents. Our overall goal is to gain insight into factors responsible for intrinsic and inducible AG resistance in the Bcc. We hypothesize that unique properties of the *Bv* OM confer AG susceptibility.

Methods

11 Bv isolates and 1 CF B. cenocepacia isolate were chosen for further study. Bv isolates represent environmental, non-CF clinical and CF isolates, including CF serial isolates of strains that acquired AG resistance. MALDI-TOF mass spectroscopy (MS) was used to analyze isolate lipid A structure. OM permeability to gentamicin, an AG antibiotic, was evaluated with the fluorescent probe 1-N-phenylnapthylamine (NPN).

Results

MALDI-TOF MS revealed no lipid A structural differences between the isolates at sites associated with AG entry. No permeability differences to NPN following gentamicin treatment were observed between the isolates.

Conclusion

These data suggest that in Bv AG entry does not occur via self-promoted uptake. Future work includes permeability assays to labeled gentamicin, OM protein analysis and evaluation of active efflux as a potential AG resistance mechanism in the Bcc. This study may aid in the improved treatment of Bcc infections.

Varun Saran[1], Ramesh Saeedi[2], Richard Wambolt[1] and Michael F. Allard[1]

[1]Dept of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada. [2]Healthy Heart Program, University of British Columbia, Vancouver, British Columbia, Canada

Varun Saran

Control of glycolysis in cardiac hypertrophy: interaction of hexokinase and voltage dependant anion channel

Backround/Objectives

Glycolysis is accelerated in pathologic cardiac hypertrophy but the cellular mechanism(s) responsible are not yet fully understood. Binding of hexokinase (HK), a key enzyme in control of glycolysis in heart and other tissues, to mitochondrial voltage dependent anion channels (VDAC) is viewed as a major factor responsible for insulin-induced glucose uptake in skeletal muscle and acceleration of glycolysis in malignant neoplasms. Thus, we tested the hypothesis that binding of HK to VDAC is increased in pathologically hypertrophied heart muscle cells and hearts..

Methods

Binding of HK to VDAC was assessed by co-immunoprecipitation and immunoblot analysis of lysates from cultured H9c2 cells hypertrophied by exposure to arginine vasopressin (AVP) and from hypertrophied hearts in mice with an abdominal aortic constriction (AAC). Cells unexposed to AVP and sham-operated mice served as Controls.

Results

AVP-treated H9c2 cells and hearts from mice with an AAC were hypertrophied 20 to 30% with rates of glycolysis 30 to 50% greater than corresponding Controls. Co-immunprecipitation experiments indicated that association of HK with VDAC was significantly increased in hypertrophied H9c2 cells and in hypertrophied mouse heart tissue.

Conclusion

These results provide support for the concept that binding of HK to VDAC is increased in the setting of pathologic cardiac hypertrophy and may play a role in the acceleration of glycolysis observed.

David Marchant

David Marchant, Gurpreet Singerha, Jonathan Boyd, Zongshu Luo, Xioaning Si, Delbert R. Dorscheid, Bruce M McManus and Richard G Hegele

Dept of Pathology and Laboratory Medicine, UBC

P38 MAP kinase is activated by toll like receptor 4 via MyD88 during virus entry to activate virus internalisation and replication

Respiratory viruses continue to exert a heavy toll of morbidity and mortality worldwide. There is the lingering threat of an avian influenza pandemic and hospitalisations due to RSV are ever increasing. Despite this grim reality there are few specific treatments for RSV infection and many influenza isolates are now resistant to amantidine and TamifluTM. We screened a panel of small-molecule kinase inhibitors to determine the most pertinent host cell kinase during virus infection. We identified p38 and ERK Mitogen Activated Protein (MAP) kinases as the most sensitive targets, such that inhibition of these kinases resulted in significant inhibition of all four Adeno, Paramyxo, Picorna, and Orthomyxo virus families investigated. MAP kinase activation is biphasic and we show that early activation of p38 is required to activate virus entry trafficking and internalisation. Virus interaction with the host-cell's own toll like receptor type 4 (TLR4) activated p38-mediated virus internalisation via a MyD88-dependent signalling pathway, in all 4 virus families. The resulting p38 activation is required for virus localisation to perinuclear regions as determined by single virus particle confocal microscopy and 3D reconstruction. Finally we demonstrate the promise of p38 as an effective antiviral approach, in-vivo. Therefore p38 MAP kinase inhibition is a highly efficacious and broad antiviral approach.

S.P. Shah[1], R. Morin[2], J. Khattra[1], L. Prentice[1], T. Pugh[2], A. Burleigh[1], A. Delaney[2], K. Gelmon[3], R. Guliany[1], R. A Holt[2], S. Jones[2], M. Sun[1], R. Moore[2], A.E. Teschendorff[4], K. Tse[1], G. Turashvili[1], R. Varhol[2], R.L. Warren[2], P. Watson[5], Y. Zhao[2], C. Caldas[4], D. Huntsman[6], M. Hirst[2], M.A. Marra[2], S. Aparicio[1]

[1]Molecular Oncology, BC Cancer Research Centre, [2]Michael Smith Genome Sciences Centre, BC, [3]Medical Oncology, BC Cancer Agency, [4]Cancer Research UK, Cambridge Research Institute, [5]Deeley Research Centre, [6]Centre for Translational Genomics

Sohrab Shah

Mutational evolution of a lobular breast tumour, profiled by whole-transcriptome and whole-genome next generation sequencing

Backround/Objectives

The accumulation of genome aberrations over time and after therapy is of relevance to the initiation and progression of breast tumours and to patient outcomes. Until recently it has not been possible to address at the single nucleotide level, the fundamental question, how many and what type of mutations occur during multi-year tumour progression?

Methods

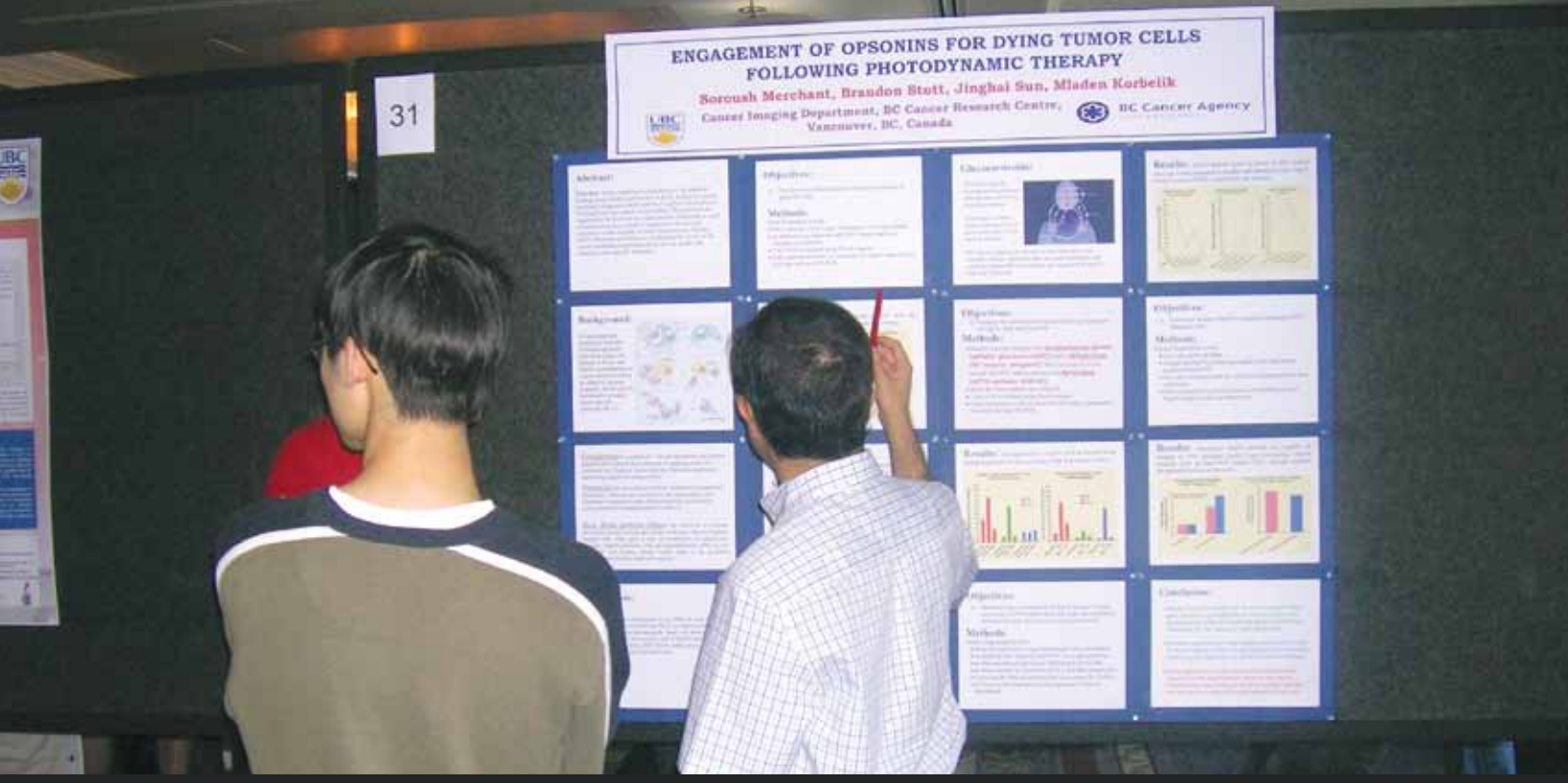
Here we describe the results from massively parallel sequencing of the transcriptomes and genomes of an estrogen receptor positive metastatic lobular breast cancer and the genome sequence of the primary tumour in the same patient, diagnosed 9 years earlier.

Results

Our combined sequence analyses captured the majority of coding mutations and provided an integrated portrait of tumour pathophysiology, revealed in alternative splicing of core estrogen signalling pathway components and the insulin receptor and also the expression biases of the tumour genome somatic mutations. Our analysis revealed RNA editing resulting in non-synonymous substitution in two transcripts, encoding SRP9 and COG3. Sequence derived copy number analysis of the primary and metastatic genomes, showed the presence of a novel amplicon containing the insulin receptor locus, which we found present in 25% of lobular breast carcinomas, suggesting this as a mechanism contributing to INSR over expression. We observed 21 somatic single nucleotide coding mutations from the combined metastasis sequencing, 3 of which were also present in the primary tumour, with an additional 18 mutations observed exclusively in the metastasis.

Conclusion

This indicates human breast cancer can be initiated by a relatively small number of driver mutations and supports the concept that metastases arise from highly evolved derivative subclones.



Poster Presentations

Vucic EA, Wilson IM, Coe BP, Chari R, Lam S, Lam WL

British Columbia Cancer Research Centre, Vancouver, Canada

Emily Vucic

Abstract # 28

The role of DNA methylation in development of lung cancer in former smokers

Backround/Objectives

Lung cancer is the most common cause of cancer death worldwide with more than 1.2 million people dying of the disease each year. Half of newly diagnosed lung cancer patients are former smokers. Understanding why former smokers develop lung cancer is clearly important to the development of early detection, prevention and treatment strategies for these people. The effects of cigarette smoke on the epigenome are widespread as both global DNA methylation and local DNA methylation have been identified, associated with genomic instability and tumour suppressor gene (TSG) silencing, respectively. In a cancer-specific context, upregulation of oncogenes and silencing of tumour suppressor genes both occur as a result of tobacco smoke exposure. Therefore, molecular studies examining tumors at genomic and epigenomic levels will likely identify causal genetic events involved in cancer development in former smokers. Objective: The objective of this study is to determine the contribution of DNA methylation as a mechanism to lung cancer development in former smokers.

Hypothesis: As smoking induces methylation changes in bronchioepithelial cells, we hypothesize that this constitutes the first hit to TSG inactivation. In order to inactivate both alleles, these methylation changes would be maintained in tumors where a second hit would be found at the same gene loci.

Methods

Epithelial cells from former smokers (those with >10 years of smoking cessation) were collected from peripheral airways during routine bronchoscopy. Half of the cells were fixed in Cytolyt and the other half in RNAlater for DNA and RNA extraction, respectively. Copy number profiling was performed by array comparative genomic hybridization (aCGH) using whole-genome tiling path SMRT v2 BAC array. Methylation analysis was performed by coupling affinity based enrichment of methylated sequences with hybridization to the same aCGH platform described above. Expression status of genes was determined by gene expression microarray analysis using Agilent 44K expression arrays.

Results

Preliminary MeDIP aCGH of bronchial brush cells from eight former smokers who had previous surgical removal of Stage I NSCLC, and eight former smokers without NSCLC, of similar age (68±7 versus 62±6), revealed distinct differences in frequency of DNA methylation between cancer and non-cancer groups. For example, at 11p13 the cancer group shows hypermethylation at the WT1 locus, a known TSG. Analysis in 62 NSCLC tumors for gene dosage, showed a high frequency of loss at the WT1 locus. To examine downstream effects of these events in tumours, expression of WT1 was assessed and found to be significantly underexpressed in the majority of NSCLC tumours compared to a normal lung reference. Collection of more samples and further integrative analysis is currently underway.

Conclusion

Differences in methylation between these two groups may explain why some former smokers develop cancer while others remain cancer free despite similar lifestyle changes. As methylation is a reversible DNA modification, this knowledge would prompt the development and application of DNA demethylation chemopreventative agents and unique therapeutic strategies.

Abstract # 29

Emily Vucic

Emily A. Vucic, Timon P.H. Buys, Raj Chari, Carolyn Brown, Calum MacAulay, Wan L. Lam

British Columbia Cancer Research Centre, Vancouver, Canada

Methylation as a mechanism of multidrug resistance in ovarian carcinoma

Backround/Objectives

Understanding the mechanisms contributing to drug resistance is crucial to cancer treatment. It is well documented that gene amplification occurs as a result of exposure to chemotherapeutic agents. Isolation of DNA in resistant cells has led to the identification of genes that confer the multi-drug resistance (MDR) phenotype. Hypothesis: We hypothesize that a change in gene dosage is not the only mechanism conferring resistance. Epigenetic changes in methylation status such as hypomethylation may also increase transcription of genes contributing to drug resistance. Objective: To identify novel genes driving the MDR phenotype by integrative analysis of whole genome DNA copy number, methylation and expression profiles from a vincristine sensitive ovarian carcinoma cell line (SKOV3) and its MDR derivatives (SKVCRs).

Methods

Segmental copy number profiles were generated utilizing the whole genome tiling path comparative genomic hybridization (CGH) array platform. Whole genome methylation profiles were determined by Methylated DNA Immunoprecipitation (MeDIP) array CGH. The same technique was applied to the Agilent 244K CGH oligonucleotide array to resolve regions of differential methylation. To demonstrate concomitant alterations in expression, whole genome expression analysis was performed using Affymetrix U133+2 expression arrays. Alignment and statistical analysis of genomic, epigenomic and expression profiles was achieved using customized SIGMA2 and SeeGH software.

Results

Characterization of whole genome array CGH and methylation profiles for SKOV3 and SKVCRs revealed distinct regional copy number alterations, including previously characterized genes involved in drug resistance, such as several ECM and ABC transporter genes. Interestingly, our data showed differential regions of hypomethylation corresponding to loci of DNA copy number amplification and analysis confirmed the downstream effects of these alterations at the DNA level. We also found hypermethylated regions and concomitant copy number loss corresponding to decreases in expression of specific genes. Therefore changes in methylation and copy number status may synergistically contribute to the drug selection response. Remarkably, alteration in methylation status of some regions corresponded to expression changes in the absence of copy number alteration, suggesting that for some genes methylation may be the primary mechanism underlying expression changes that may contribute to chemoresistance.

Conclusion

We have identified genomic, epigenetic alterations that emerge with increasing drug selection in an ovarian carcinoma cell line using a unique integrative and whole genome approach. These alterations may help to decipher the genes involved in acquiring or maintaining the MDR phenotype in ovarian cancer.

Cherry Ma[1,2], Jas Khattra[1,2], Angela Burleigh[1,2], Samuel Aparicio[1,2]

[1]Department of Pathology, University of British Columbia, [2]Department of Molecular Oncology and Breast Cancer, BC Cancer Research Centre

Cherry Ma

Study of genetic hierarchical lineage of breast cancer tumor cells

Backround/Objectives

Tumor cell heterogeneity has been suggested by evidence from various tumor systems. It is believed that tumor mass develop from a few number of rare tumor initiating cells that possess stem cell property of proliferating and differentiating. As cells replicate, genomic aberrations accumulate and contribute to the cell transformation. The concept of the cancer stem cell might be the reason of tumor relapse and metastasis after cancer treatment. Sequencing genomes of both primary and metastasized lobular breast cancer tumor from the same patient gives a list of potential genes involved in tumor transformation. We hypothesize that there is a genetic hierarchical lineage existing in cancer cells genomes in a tumor mass.

Methods

By introducing genomic aberrations one by one in hTERT immortalized normal breast cells; we may be able to transform normal cells into tumor cells and revealing the genetic hierarchical lineage in the process. In order to do so, we will first analyze the frequency of genomic aberrations in the tumor by doing single cell QPCR analysis on micro-fluidic chip for genes identified in the breast tumor-sequencing project. Based on the QPCR data, we will introduce genomic mutations into hTERT immortalized diploid normal breast cells using zinc finger nuclease mediated site-specific homologous recombination. The genomic aberration will be accumulated by introducing the mutations one by one and the phenotype of cells with mutated genomes will be characterized. The site-specific zinc finger nuclease will be constructed by applying OPEN (Oligomerized Pool Engineering) selection methods developed by Zinc Finger Consortium.

Results

Based on the analysis of data from the single cell QPCR and genomic modification, we hope to reconstruct the genetic hierarchical lineage.

Abstract # 31

Jennifer Y. Kennett

Jennifer Y. Kennett[1, 2], Stephanie Yee[3], Wan L. Lam[1,2]

[1]Dept of Pathology, University of British Columbia, Vancouver B.C., Canada, [2]B.C. Cancer Research Centre, Vancouver B.C. Canada, [3]Princess Margaret Hospital, Toronto O.N., Canada

Characterization of genomic alterations in a subset of retinoblastoma tumors using high resolution array comparative genomic hybridization

Backround/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 about 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 genetic alteration 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 copy number variations to provide insight into the underlying biology of these tumors.

Methods

A sample set composed of DNA isolated from 14 NMF and 15 RB1-/- Retinoblastoma tumors was obtained from Dr. Brenda L. 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 previously unachievable resolution. Array data was assessed using custom SeeGH software and regions of copy number alteration were defined and compiled for each sample.

Results

Whole genome array CGH profiles were generated for all 14 NMF and 15 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, and subsequently may lead to the development of new targets for diagnosis in this subset of patients.

Kelsie L. Thu[1], William W. Lockwood[2,3], Raj Chari[2,3], Bradley P. Coe[3], Calum MacAulay[2,3], Luc Girard4, Adi F. Gazdar[4], John D. Minna[4], Stephen Lam[3], Wan L. Lam[2,3]

[1]UBC, Interdisciplinary Oncology Program, [2]UBC, Department of Pathology and Laboratory Medicine, [3]British Columbia Cancer Research Centre, [4]Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center Dallas, TX

Kelsie Thu

Identification of candidate tumour suppressor genes in lung cancer by integrative genomic analyses

Backround/Objectives

Inactivation of tumour suppressor genes (TSGs) like p53 is characteristic of lung cancer, and leads to cellular transformation. Homozygous deletion, the loss of both alleles of a gene, is one genetic mechanism for inactivating TSGs. These events occur due to strong selection, making them excellent markers for identifying important TSGs that may also be silenced by other mechanisms. Discovery of TSGs and the consequences of their inactivation has revealed signalling pathways commonly disrupted in lung cancer, enhancing our understanding of its development. Identification of these TSGs will contribute to our understanding of and ability to prevent and treat lung cancer. The aim of this study was to discover candidate TSGs inactivated by DNA deletion in lung cancer using an integrative genomics approach.

Methods

Copy number profiles of 86 lung cancer cell lines were generated by whole genome tiling path array comparative genomic hybridization. Regions of deletion and loss were systematically determined for each sample and compiled across all genomes analyzed. Expression profiles were generated for 66 of the cell lines using the Affymetrix U133A platform for integration with genomic data. Identified genes were separated based on copy number status (deletion versus neutral) and their expression compared using the Mann Whitney U test to evaluate the effects of deletion on transcript levels. Candidate genes were validated by investigating copy number and expression levels in a panel of NSCLC samples.

Results

Array CGH revealed both reported and novel regions of frequent deletion. 232 'hotspots' of homozygous deletion were identified which were altered in >5% of the samples analyzed. Chromosome 18 showed a relative enrichment for deletions suggesting it may harbour several TSGs, while chromosomes 7 and 14 had the fewest. Known TSGs including SMAD4, FHIT, and CDKN2A were lost or homozygously deleted in a large proportion of cases validating our approach. Integration of copy number and expression data revealed significantly lower expression in samples with deletions versus those without for 95 genes within the deletion hotspots. Among these are lung candidate TSGs such as WWOX and PTPRD, which further validates the utility of our integrative method in TSG discovery. We identified homozygous deletion as a novel genetic mechanism of inactivation of the candidate TSG SMARCA2, which has been shown to be silenced by epigenetic alterations in lung cancer. In addition, we identified KLHL9 (9p) and MYOM2 (8p) as two novel candidate TSGs frequently inactivated in lung cancer. These genes require further functional analysis to validate their tumour suppressor potential.

Conclusion

We have identified both reported and novel candidate TSGs in lung cancer, including WWOX and SMARCA2, and KLHL9 and MYOM2, respectively. Functional validation is required to investigate the tumour suppressor potential of the genes identified and these findings may provide novel targets for lung cancer diagnosis and treatment.

Abstract # 33

Kelsie Thu

Kelsie Thu[1,3], William W. Lockwood[2,3], Ariane C. Williams[3], Andy Lam[3], Wan L. Lam[2,3]

[1]University of British Columbia, Interdisciplinary Oncology Program, [2]University of British Columbia, Department of Pathology and Laboratory Medicine, [3]British Columbia Cancer Research Centre

Discovering oncogenes in pancreatic cancer using integrative genomic analyses

Backround/Objectives

Pancreatic ductal adenocarcinoma (PDAC) ranks among the top five deadliest cancers in the world. The dismal prognosis for this cancer is directly attributable to the lack of early detection methods and effective treatment options. PDAC-specific genetic amplifications may harbour oncogenes important to cancer progression, and represent potential therapeutic targets in pancreatic cancer. The goal of this study is to identify novel genetic amplifications in PDACs that contain candidate genes in pancreatic cancer development by integrating copy number and gene expression data.

Methods

A panel of 20 pancreatic cancer cell lines was studied. Genetic profiles displaying gene dosage alterations were obtained for each cell line by comparative genomic hybridization (CGH) using a whole genome tiling-path array. Genomic regions with significantly frequent, high level amplifications across the PDAC cell lines were identified using the GISTIC analysis method. These regions were further investigated to determine the effects of increased gene dosage on gene expression. Agilent whole genome expression arrays were used to generate mRNA transcript profiles. Genes located within the discovered amplicons were examined for associations between copy number status and gene expression by comparing expression levels between samples with and without copy number gains and by direct correlation analysis.

Results

CGH analysis revealed the presence of multiple copy number gains and losses in different chromosomal regions specific to each PDAC cell line as well as recurrent changes present in multiple cell lines. GISTIC analysis revealed 27 significantly amplified regions of DNA ($p=0.05$), of which, 14 have not been previously reported. Within these amplicons, several candidate genes were identified as having correlated increases in copy number and gene expression, demonstrating that increased gene dosage is a mechanism of gene activation in PDAC.

Conclusion

Novel genetic amplifications were identified in PDAC cell lines, and the increase in gene dosage of several genes within the discovered amplicons appears to have an effect on their expression levels. The candidate genes within these regions warrant functional analyses to determine their roles in the development and progression of pancreatic cancer.

K Schrader[1,3], S Masciari[2], J Senz [1], N Tung[4], J Balmana[5], J Garber[2], D G Huntsman[1,3], Breast Cancer Family Registry (B-CFR) and the BCRF Breast Cancer Genetics Consortium[6]

[1]Dept of Pathology and Laboratory Medicine, UBC, Vancouver, BC, [2]Dept of Medical Oncology, DFCI, Boston, MA, [3]Hereditary Cancer Program, BCCA, Vancouver, BC, [4]Beth Israel-Deaconess Medical Center, Boston, MA, [5]Hospital Vall d'Hebron, Barcelona, Spain, [6]Breast Cancer Research Foundation, NY

Kasmintan Schrader

Early onset or familial lobular breast cancer and the association of germline CDH1 mutations

Backround/Objectives

Invasive lobular breast carcinoma (LBC) has been identified as a component of the hereditary diffuse gastric cancer (HDGC) syndrome, associated with germline mutations in the E-cadherin (CDH1) gene. The estimated risk of lobular breast cancer to age 80 in female CDH1 mutation carriers is 39-50%: the risk of diffuse gastric cancer is 67% in males, and 83% in females. Management of HDGC includes prophylactic gastrectomy. CDH1 mutations can be identified in around 50% of HDGC families from populations of low gastric cancer incidence. In this study, we aimed to estimate the prevalence of germline CDH1 mutations among women diagnosed with early onset LBC or family history of breast cancer.

Methods

Germline DNA was collected from 384 women with lobular or mixed lobular and ductal breast cancer from breast cancer programs, familial cancer clinics, and population-based cohorts. Women with known germline BRCA1 or BRCA2 mutations were excluded. Eligible women had (1) LBC before age 45 years or (2) LBC with at least two 1st or 2nd degree relatives with breast cancer independent of age at diagnosis. Denaturing high pressure liquid chromatography was undertaken, followed by direct sequencing of exons displaying changes.

Results

Of 369 samples analyzed for heterozygous sequence variants, one previously characterized mutation and five novel non-synonymous variants (1.9%) were found. Three of these women had LBC before 45 years of age and no family history of breast cancer; two had breast cancer family history. One novel non-synonymous change was found in two unrelated families. Haplotype data suggests that this could represent an ancient non-synonymous variant. No gastric cancers were reported in these families. Functional assays to assess the pathogenicity of the variants are in process.

Conclusion

Results from this large cohort confirm that CDH1 is responsible for at most a small proportion of familial and early onset LBC. In light of the known association with HDGC and LBC, these findings indicate that without a family history of gastric cancer, the likelihood of finding a germline CDH1 mutation in a proband with early onset LBC or LBC accompanied by a family history of breast cancer, is low. Thus obtaining extensive family histories for gastric cancer will be crucial in determining which LBC families to offer germline CDH1 mutation testing to. Additionally, in view of the previous LBC family reported, a family history of the lobular breast cancer subtype exclusively occurring at a young age may also be an indication for CDH1 germline mutation testing.

Abstract # 35

Kimberly Wiegand

Kimberly C. Wiegand[1], Sohrab P. Shah[1], Ryan D. Morin[3], Gillian Leung[1], Mark Sun[1], Ryan Guiliany[1], Martin Hirst[3], C. Blake Gilks[2], Marco Marra[3], David G. Huntsman[1,2]

[1]Centre for Translational and Applied Genomics of British Columbia Cancer Agency and the Provincial Health Services Authority Laboratories, Vancouver, BC, [2]Genetic Pathology Evaluation Centre of the Depts of Pathology of the VGH, British Columbia Cancer Agency and the UBC and the Prostate Research Centre, Vancouver BC, [3]Genome Sciences Centre, British Columbia Cancer Agency, Vancouver BC

Redefining ovarian cancer subtypes with paired end whole transcriptome shotgun sequencing (PEWTSS)

Backround/Objectives

The ovarian carcinoma subtypes include low-grade serous, high-grade serous, clear cell, mucinous, and endometrioid carcinomas. These pathologically identifiable subtypes are essentially different diseases, having different precursor lesions, biomarker profiles, clinical behaviors, and response to treatment. The objective of this project is to characterize the transcriptomes of clear cell and endometrioid carcinomas using PEWTSS, with the goal of finding subtype specific early detection markers and potential drug targets. As both of these cancer types are associated with endometriosis, we also hope to identify mutations in DNA repair and other pathways that explain these divergent phenotypes.

Methods

Solexa/Illumina whole-transcriptome resequencing is being used as a platform for discovery of potential fusion gene candidates, transcript abundance, novel SNVs, and indels. Unlike traditional sequencing methods which rely on dye terminator technology and the generation of one specific gene or transcript sequence at a time using capillary electrophoresis, Illumina sequencing technology provides a cost effective approach to rapidly produce billions of bases of sequence data. Traditional Sanger sequencing is used to validate potential mutations, and fluorescent in situ hybridization (FISH) is used to validate promising candidate fusion genes on 445 samples archived with the BC Ovarian Tumor Bank.

Results

To date the transcriptomes of five clear cell carcinomas have been sequenced with PEWTSS, with the identification of 501 potential gene fusions, 97 indels, and 3995 novel SNVs. Four endometrioid carcinomas have been sequenced, with the identification of 62 potential gene fusions, 219 indels, and 2619 novel SNVs. Expected mutations in the in the beta-catenin gene in the endometrioid cases have been confirmed, as have known mutations in the other ovarian carcinoma subtypes, allowing for the validation and improvement of the Solexa/Illumina data processing pipeline. Novel mutations in other candidate genes of interest are being prioritized and validated using Sanger sequencing.

Conclusion

In addition to the previously identified and published mutations in clear cell and endometrioid carcinomas, novel subtype specific mutations in DNA repair genes, kinases, and other potential drug targets have been identified. The discovery of such subtype specific abnormalities in these ovarian carcinomas could radically change the way these diseases are diagnosed and potentially treated.

Cheang M.C.U[1], Chia S.K.[2], Tu D.3, Jiang S.[3], Choo J.R.[1], Levine M.N.[3], Shepard L.E.[3], Pritchard K.I.[4], Nielsen T.O.[1]

[1]University of British Columbia, Vancouver BC, [2]BC Cancer Agency, Vancouver BC, [3]NCIC Clinical Trials Group, Kingston ON, [4]University of Toronto, Toronto ON

Jennifer Choo

Abstract # 36

Anthracyclines in basal breast cancer: the NCIC-CTG trial MA5 comparing adjuvant CMF to CEF

Backround/Objectives

MA5 is a Canadian breast cancer adjuvant therapy clinical trial which randomized premenopausal women with node-positive early breast cancers to cyclophosphamide-epirubicin-fluorouracil (CEF) or cyclophosphamide-methotrexate-fluorouracil (CMF) adjuvant chemotherapy. This and other trials have shown that adjuvant regimens containing anthracyclines confer significant survival benefit to breast cancer patients, and has lead to their widespread adoption, despite major side effects. Meta-analyses have revealed most benefit is conferred to women with HER2(+) tumors. Previous work on patients from British Columbia found that breast cancers with a core basal phenotype (negative for hormone receptors and HER2, positive for CK5/6 or EGFR) seemed to have worse survival on anthracycline-containing versus older CMF regimens. Here we use tissue samples from the MA5 randomized trial to test the hypothesis (specified a priori) that for basal breast cancers anthracyclines may be inferior, using data from MA5.

Methods

From the 710 patients enrolled in MA5, blocks suitable for tissue microarray construction were recovered for 549. Slides were reviewed and areas of invasive carcinoma circled for tissue core extraction. Immunohistochemistry for ER, PR, HER2, Ki67, CK5/6 and EGFR was performed and scored by prespecified methods, allowing stratification of 511 cases into intrinsic biological subtypes. Survival analyses were conducted independently by the NCIC-Clinical Trials Group statistical centre.

Results

14% of women in the trial had a core basal phenotype. In the CEF (anthracycline) arm, patients with core basal tumors had a hazard ratio of 1.8 (log-rank $p = 0.02$) for overall survival relative to the other biological subtypes. In the CMF arm, there was no significant difference (HR 0.9, $p = 0.7$). The interaction between core basal status and treatment was borderline significant ($p = 0.06$). In contrast, HER2-expressing subtypes have a 25% improvement in overall survival with anthracyline treatment, which is consistent with previous findings.

Conclusion

Data from this randomized trial supports the hypothesis that anthracycline-containing adjuvant chemotherapy regimens are actually inferior to older, less-toxic CMF regimens in women with basal breast cancer.

Abstract # 37

Paul Hiebert

Paul Hiebert[1,2], Thomas Abraham[1], Wendy Boivin[1,2], Hongyan Zhao[1], David Granville[1,2]

[1]Providence Heart + Lung Institute at St. Paul's Hospital, [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Granzyme B in skin aging and extracellular matrix degradation in apolipoprotein E deficient mice

Backround/Objectives

Granzyme B (GrB) is a serine protease stored within cytotoxic granules of cytotoxic lymphocytes and is involved in immune-targeted cell death. GrB can function intracellularly to cause apoptosis and can also act extracellularly by cleaving extracellular matrix (ECM) proteins and has been implicated in several inflammatory diseases. We and others have observed an inflammatory skin aging phenotype in apolipoprotein E (ApoE) deficient mice, an established mouse model of atherosclerosis, and that this phenotype is accelerated when mice are fed a high fat diet. The purpose of the current study is to characterize the ApoE knockout (KO) mouse as a model of accelerated aging and to determine the role of GrB in this process.

Methods

Wild type C57BL/6, ApoE-KO and ApoE/GrB double knockout (DKO) mice were grown to 5, 15, 30 or 60 weeks on either a regular chow diet or a high fat diet. H&E and picrosirius red stains were used to assess morphology and collagen content in formalin fixed skin sections. Multi-photon microscopy was also used to examine collagen levels and morphology in ex vivo mouse skin samples.

Results

ApoE-KO mice show signs of frailty, hair loss and reduced longevity compared to wild type controls. H&E staining shows a clear reduction in subcutaneous adipose tissue along with increased inflammation and evidence of xanthomatous lesions in ApoE-KO mice compared to controls. Picrosirus red staining reveals differences in the collagen I and collagen III content of the skin of ApoE-KO mice compared to wild type controls. As shown using multi-photon microscopy, 60 week old wild type C57 mice on a high fat diet gave a collagen signal nearly twice as strong as the high fat diet fed ApoE-KO mice at 30 weeks. At 60 weeks, the ApoE/GrB DKO mouse skin gave a collagen signal even greater than that of the wild type mouse.

Conclusion

These results suggest that hypercholesterolemia accelerates aging of the skin in ApoE-KO mice and that GrB contributes through the modification and degradation of ECM proteins. Models and techniques successfully used here may also help toward future studies looking at ECM changes in vascular aging in the skin and other tissues.

Lisa S. Ang, Ciara M. Chamberlain, Sarah J. Williams, Wendy A. Boivin, Hongyan Zhao, Michael F. Allard, Bruce M. McManus, David J. Granville

The James Hogg iCAPTURE Centre, Providence Heart + Lung Institute, Department of Pathology and Laboratory Medicine, University of British Columbia

Lisa Ang

Extracellular activity of granzyme B contributes to abdominal aortic aneurysm and rupture

Backround/Objectives

Abdominal aortic aneurysm (AAA) is an age-related disease caused by progressive weakening of the vessel wall. Although it is often fatal, effective pharmacological interventions aimed at halting AAA progression at early stages of disease are not available. Previous work in our laboratory has demonstrated a role for the serine protease Granzyme B (GrB) in the development of AAA; however, the mechanism by which this occurs is unclear. GrB is better known for its role in eliminating target cells via apoptosis, but also accumulates extracellularly during inflammatory states and has been shown to cleave extracellular matrix components such as fibronectin, vitronectin and laminin. Therefore, we hypothesize that the inhibition of extracellular GrB will prevent the development of AAA.

Methods

Because GrB requires perforin to gain entry into cells and initiate apoptosis, GrB-/- x apoE-/- (GDKO) and perforin-/- x apoE-/- (PDKO) mice were generated to determine whether GrB affects AAA development via intracellular or extracellular activity. To induce aortic aneurysm, GDKO, PDKO and apoE-/- mice were implanted with an osmotic minipump that released angiotensin II for 28 days. A second group of apoE-/- mice were also injected with either a serpin inhibitor of GrB (apoE/Ser) or saline (apoE/Sal) in addition to the pump. Mice were euthanized and tissues harvested after 28 days.

Results

Reduction in AAA was observed in the GDKO and apoE/Ser mice compared to PDKO, apoE-/- and apoE/Sal groups. Aneurysms observed in GDKO and apoE/Ser mice were also smaller and less likely to rupture. GrB deficiency or inhibition also corresponded with a substantial increase in survival at 28 days and prevented the loss of fibrillin-1 in the aorta. Because perforin deficiency did not provide any significant protective effect, it would appear that GrB contributes to AAA pathogenesis via its extracellular cleavage activity rather than the intracellular apoptotic pathway and that the serpin asserts its protective effect via the inhibition of extracellular GrB.

Conclusion

In summary, our data suggests an extracellular role for GrB in the pathogenesis of AAA.

Abstract # 39

Jon Carthy

Jon Carthy, David Marchant, Seti Boroomand, Darryl Knight and Bruce McManus

Pathology and Laboratory Medicine, The University of British Columbia/The James Hogg iCAPTURE Centre, Vancouver, BC, Canada

Versican induces fibroblast-mediated tissue remodeling

Backround/Objectives

Versican is a chondroitin sulfate proteoglycan found in the extracellular matrix (ECM) of many tissues and is a major component that accumulates in lesions of cardiac allograft vasculopathy. Versican is considered pro-atherogenic and central to vascular injury and repair events because of its ability to trap cholesterol-rich lipoproteins, in addition to its impact on cell adhesion, survival, proliferation, and migration. The current study evaluated the hypothesis that versican modifies tissue remodeling by altering fibroblast-mediated contraction of the ECM.

Methods

We have cloned human versican and set up an inducible (doxycycline controlled) expression system in mouse embryonic fibroblasts. Cells were cultured in 3D native type I collagen gels in the presence or absence of versican overexpression, and contraction was followed for a period of 24 hours.

Results

Results suggest versican significantly increased fibroblast contraction of the collagen gels (27.9 1.2% vs 64.5 1.4% of initial gel area, $p<0.01$). Immunohistochemistry and confocal imaging of these gels demonstrated a marked change in cell phenotype: versican expressing fibroblasts exhibited cellular protrusions that were associated with smooth muscle alpha-actin (SMA) positive stress fibres (a marker of myofibroblast differentiation), while non-induced cells were rounded and negative for SMA. Further, versican appeared to form a pericellular coat around these cellular protrusions suggesting it may be influencing cell receptor-to-matrix interactions. In support of this idea, Western blotting demonstrated that versican overexpression dramatically increased the expression of beta 1 integrin.

Conclusion

Taken together, our data suggests versican induces myofibroblast differentiation during tissue remodeling. If versican also promotes tissue remodeling, the relative amount of versican produced at sites of injury may be critical in regulating vessel wall geometry (shrinkage) after heart transplantation.

D. Lin[1], G.C. Freue[2], Z. Hollander[3], A. Bergman[3], M. Sasaki[3], A. Ignaszewski[4], J. Wilson-McManus[1], R. Balshaw[2], R. Ng[5], R. McMaster[6], P. Keown[4], B. McManus[1] for the Biomarkers in Transplantation Team

[1]Dept of Pathology and Laboratory Medicine, University of British Columbia, [2]Department of Statistics, UBC, [3]The James Hogg iCAPTURE Centre for Cardiovascular & Pulmonary Research, [4]Dept of Medicine, UBC, [5]Dept of Computer Science, UBC; [6]Dept of Medical Genetics, UBC

David Lin

Genomic and proteomic biomarkers of chronic cardiac rejection

Backround/Objectives

Despite the advancements in cardiac transplantation and immunosuppressants, long-term graft survival remains a problem and is largely limited by cardiac graft vasculopathy, also known as chronic rejection (CR). The current analysis focuses on biomarkers of chronic cardiac allograft rejection identified using genomic and proteomic approaches.

Methods

Serial blood samples from cardiac transplant subjects from pre-transplant through 3 years post-transplant were collected. Whole blood and plasma samples were analyzed with Affymetrix HG U133 plus 2.0 microarrays and iTRAQ proteomics, respectively. Each iTRAQ proteomic run consisted of 3 patient samples and a pooled normal control for relative comparisons. Patients were 26 to 70 years old; 2/3 were male. CR diagnoses were made based on quantitative analysis of angiograms, intravascular ultrasound and chart review. The genomics and proteomics data were analyzed using a moderated t-test.

Results

On the genomics side, 10 genes were differentially expressed between CR versus stable (S) samples with FDR <5%. Initial internal validation results revealed that the 10 genes together discriminated between CR and S samples with 83% sensitivity and specificity. On the proteomics side, 10 proteins were identified as differentially expressed between the CR and S samples ($p < 0.025$).. Of the 10 proteins, 7 are relatively well-characterized and have been linked to immune mechanisms, cell adhesion, and cell communication. Interestingly, internal validation of the proteomic CR biomarker panel also demonstrated 83% sensitivity and specificity.

Conclusion

Using cardiac recipient whole blood and plasma samples, we have identified genomic and proteomic biomarkers that can diagnose chronic cardiac rejection. These potential biomarkers will be validated in our upcoming Canada-wide trial.

Acknowledgements: Genome Canada, Novartis, IBM, Genome British Columbia, PROOF Centre of Excellence, NCE-CECR.

Abstract # 41

Guang Gao

Guang Gao, Jerry Wong, Jingchun Zhang, Jayant Shravah, Honglin Luo

The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, Providence Heart + Lung Institute, Department of Pathology and Laboratory Medicine, St. Paul's Hospital-University of British Columbia, Vancouver, BC

The role of proteasome activator REG-gamma in coxsackievirus replication

Backround/Objectives

Coxsackievirus B3 (CVB3) is the most prevalent virus associated with myocarditis and subsequently dilated cardiomypathy. The ubiquitin/proteasome system (UPS), a major intracellular protein degradation system, has been increasingly acknowledged as a key modulator in viral pathogenesis. We have previously demonstrated that proteasome inhibition reduces CVB3 replication and attenuates virus-induced myocarditis. However, we recognize the potential toxicity of general inhibition of proteasome function as a therapeutic means. In this study, we investigated the role of REG-gamma, a member of the 11S proteasome activators that promote ubiquitin-independent degradation of protein, in CVB3 replication. REG-gamma is a nuclear protein that is involved in the degradation of important intracellular proteins such as steroid receptor coactivator-3, a series of cyclin-dependent kinase inhibitors p21, p16, and p19, and tumor suppresor p53. It has been reported that REG-gamma binds to and regulates the stability and nuclear retention of hepatitis C core protein, indicating its important role in viral pathogenesis. In the present study, we have two objectives: (1) to determine the interplay between REG-gamma and CVB3 replication, and (2) to determine the mechanisms by which REG-gamma regulates CVB3 replication.

Methods

REG-gamma was overexpressed or knockdowned in cells, followed by sham or CVB3 infection with various times. Cell lysates were collected and expression of viral capsid protein VP1, REG-gamma, caspase-3, and beta-actin was analyzed by Western blot. Viral titres were determined by plaque assay. In vitro symoylation assay was performed to examine the possibility of REG-gamma sumoylation, a post-translational process usually targeting on nuclear proteins for regulating transcription and subcellular localization.

Results

We found that overexpression of REG-gamma increased viral replication; while knockdown of REG-gamma sensitized cells to CVB3-induced cell death and reduced CVB3 replication. REG-gamma expression was unchanged in the whole nuclear extracts after viral infection. However, CVB3 infection induced a redistribution of REG-gamma from triton soluble to insoluble fractions. Using in vitro sumoylation assay, we demonstrated that REG-gamma can be sumoylated.

Conclusion

CVB3 infection leads to the translocation of REG-gamma within the nucleus, likely through induction of REG-gamma sumoylation; REG-gamma plays an very important role in CVB3 infection.

Leslie YM Chin[1,3], Ynuk Bossé[3], Yuekan Jiao[3], Dennis Solomon[3], Peter D. Paré[2,3], and Chun Y. Seow[1,3]

[1]Dept of Pathology and Laboratory Medicine, [2]Dept of Experimental Medicine, [3]James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, St. Paul's Hospital / Providence Heart + Lung Institute / University of British Columbia

Leslie Chin

Myosin filament assembly in airway smooth muscle

Backround/Objectives

Understanding airway smooth muscle (ASM) contraction is vital to elucidating the mechanism of asthma pathogenesis since the ASM is responsible for the exaggerated airway narrowing seen in disease. Myosin filaments within ASM cells are capable of rapid assembly and disassembly; however, the mechanism of malleability is unclear. Filament integrity is thought to be dependent on regulatory myosin light chain (MLC) phophorylation. Stimulating ASM with acetylcholine (ACh) and potassium chloride (KCl) can provide insight into myosin filament organization. Although ACh and KCl cause contractions of similar force, ultrastructural examination indicates that ACh stimulation produces greater myosin filament assembly than KCl stimulation. This suggests that myosin filaments may not be necessary for force production. Non-filamentous force generation has been seen in non-muscle motile cells but has not been shown in smooth muscle cells. Under KCl stimulation we propose that myosin dimers produce force but are in a non-filamentous conformation. We hypothesize that the non-filamentous conformation is less stable than the filamentous conformation, and thus, more sensitive to mechanical perturbation.

Methods

Muscle was dissected from sheep trachealis and placed under a force transducer-myograph. The muscle was stimulated with either 40 mM KCl or ACh (concentration to produce equivalent force as KCl). 40mM KCl was chosen as it did not elicit neuronal ACh release, as verified with atropine. At force plateau, a short-duration, high-frequency oscillation was applied to the muscle. Decreases in force after oscillation were compared using ANOVA. ASM strips were also fixed at the force plateau in formaldehyde for ultrastructural imaging using transmission electron microscopy and frozen for analysis of myosin light chain phosphorylation.

Results

At similar force, ACh-stimulated cells have greater myosin filament density (ANOVA:$p<0.0001$). KCl-stimulated cells are less resistant to mechanical perturbation (ANOVA: $p<0.0001$) despite generating similar force and similar myosin light chain activation levels.

Conclusion

KCl-stimulated cells have fewer myosin filaments than ACh-stimulated cells but are able to produce similar force. This implies that airway smooth muscle cells are capable of producing force without myosin filaments. Also, myosin light chain phosphorylation may not be the sole determinant of myosin filament integrity.

Abstract # 43

Ashish Marwaha

Ashish Marwaha[1], Lisa Xu[1], Megan Levings[2], John Priatel[1] and Rusung Tan[1]

[1]Pathology and Laboratory medicine, BC Children's Hospital and Child and Family Research Institute, Vancouver, BC, Canada, [2]Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada

The role of treg and th17 cells in type 1 diabetes

Backround/Objectives

Type 1 diabetes (T1D) is an autoimmune disease caused by the destruction of insulin producing pancreatic Beta cells by autoreactive cytotoxic lymphocytes. Treg cells act to suppress the activation of these autoreactive lymphocytes in the periphery and so regulate immune responses and prevent autoimmunity. A newly described T cell subpopulation called Th17 cells produce interleukin-17 and act to promote the inflammatory response in autoimmune disease. Thus, Treg cells and Th17 cells act to counterbalance one another in vivo and a disruption in this balance could lead to autoimmune diseases such as T1D. Although previous studies have used the transcription factor Foxp3 as a marker for Treg cells, recent data has shown that both Treg and Th17 cells can express Foxp3. We used a memory marker (CD45RA) to identify novel subsets of Foxp3+ cells in peripheral blood. We hypothesized that there might be differences in these subsets between T1D patients and healthy controls.

Methods

Peripheral blood mononuclear cells, from ten T1D patients and healthy controls were purified using a ficoll gradient. These were then stained with a panel of fluorescently labeled antibodies (CD4, CD45RA, CD14, CD25 and Foxp3), to distinguish various white blood cell populations, including CD4+CD45RA+Foxp3+ and CD4+CD45RA-Foxp3+ subsets, by flow cytometry. A student's t-test was used to determine any statistical significance in these subsets between the T1D patients and healthy controls.

Results

We found that the proportion of CD4+Foxp3+ cells in T1D patients was increased relative to healthy controls. This increase was confined the subset lacking CD45RA expression.

Conclusion

There is an increase in the CD4+ CD45RA- Foxp3+ subset in T1D patients. Recent work has demonstrated that this subset can produce Il-17. This suggests the possibility that T1D patients may have more Th17 cells than healthy controls. This hypothesis will form the basis for future work.

I-Fang Lee, John Priatel, Peter van den Elzen and Rusung Tan

Departments of Pathology & Laboratory Medicine and the Child and Family Research Institute, British Columbia's Children's Hospital and University of British Columbia, Vancouver, Canada

I-Fang Lee

Natural killer T (NKT) cells and natural killer (NK) cells are required for complete Freund's adjuvant-mediated prevention of diabetes

Backround/Objectives

Type 1 diabetes (T1D) is an autoimmune disease characterized by immunological destruction of insulin-producing pancreatic beta-cells and subsequent hyperglycemia. The development of T1D is influenced by multiple genetic and environmental factors, most of which remain unknown. The nonobese diabetic (NOD) mouse strain spontaneously develops a disease similar to human T1D as a consequence of the loss of basic tolerogenic processes that control self/nonself discrimination. Our prior reports have shown that the administration of complete Freund's adjuvant (CFA) to NOD mice prevents the accumulation of beta-cell-specific cytotoxic T lymphocytes (CTL) and that protection from disease was mediated by a subset of natural killer (NK) cells. However, the mechanism(s) by which CFA activates and recruits NK cells to protect NOD mice from T1D remains unclear. Based on findings that NK cell function, including proliferation, IFN-gamma production, and cytotoxicity, may be regulated by effector cytokines produced by activated NKT cells, we sought to investigate whether NKT cells are involved in NK cell activation by CFA treatment.

Methods

6-8 week-old C57BL/6 mice, CD1d knockout mice, MyD88 knockout mice or NOD mice were given a single injection of CFA or IFA emulsion sub-cutaneously in the base of their tails. Liver and spleen were subsequently harvested at indicated time points and the frequency and function of NK or NKT cells were analyzed using Flow Cytometry. To assess the development of autoimmune diabetes, 6-8 week-old NOD and NOD CD1d knockout mice were injected a single dose of CFA emulsion or PBS. Blood glucose was monitored once weekly using test strips. Mice with a blood glucose measurement of greater than 33 mM were considered diabetic.

Results/Conclusions

We observe that NKT cell activation occurs rapidly after CFA administration through a MyD88-independent mechanism. In addition, CFA administration causes a rapid change in NK cell frequencies in the spleen and liver of wild type mice whereas no such changes are found in NKT cell-deficient CD1d-knockout mice. To determine whether NKT cells function in CFA-mediated protection of NOD mice from T1D, young wild type and CD1d-deficient NOD mice were treated with CFA and monitored for the onset of T1D. The observation that CFA failed to block development of T1D in CD1d-deficient NOD mice argues that NKT cells are essential for CFA-mediated protection from diabetes. Collectively, our studies suggest that NKT cells, in addition to NK cells, play pivotal roles in CFA-mediated protection of NOD mice from T1D.

Abstract # 45

Nga Ting Colette Chiu

Nga Ting Colette Chiu[1], Emma Tomlinson Guns[2], William Jia[3]

[1]Department of Pathology and Laboratory Medicine, University of British Columbia, Canada, [2]Department of Urologic Sciences, University of British Columbia, Canada, [3]Department of Surgery, University of British Columbia, Canada

Ginsenosides and ginsenoside metabolites affect the expression of cytochrome P450 3A4

Backround/Objectives

Ginseng and its active ingredients, ginsenosides, are used widely as complementary and alternative medicines. However, their potential effects on the metabolism of concurrently used drugs are yet to be determined. Changes to the expression of one of the most important drugs and xenobiotics metabolizing enzyme, cytochrome P450 3A4(CYP3A4), may lead to clinically significant herb drug interaction. We hypothesize that ginsenosides and their bacterial fermentation products, ginsenoside metabolites, affect the expression of the intestinal CYP3A4.

Methods

Naturally occurring ginsenosides and ginsenoside metabolites were assayed for their effect on CYP3A4 at the RNA, protein, and enzymatic levels. After Caco2 cells were treated with ginsenoside Rg1, CYP3A4 mRNA and protein expression profiles were examined by qRT-PCR and western blot, respectively. Total ginsenosides, Rg1, and ginsenoside metabolites, aPPD and aPPT were tested for their abilities to inhibit CYP3A4 enzymatic activities in vitro using dibenzylfluorescein as a probe.

Results

Rg1 inhibits CYP3A4 mRNA expression (40%) at low concentration (0.5μM) and induces CYP3A4 mRNA expression (60%) at high concentration (2 μM). Similarly, Rg1 inhibits CYP3A4 protein expression (30%) at low concentration (1μM) and induces CYP3A4 protein expression (70%) at high concentration (10μM). Total ginsenosides, aPPD, and aPPT are potent CYP3A4 enzyme inhibitors with IC50 of 37.06μg/mL, 13.77μM, and 2.35μM, respectively, whereas Rg1 does not inhibit CYP3A4 enzymatic activities.

Conclusion

Our results suggest that ginsenoside Rg1 regulates CYP3A4 mRNA and protein expressions in a biphasic manner, while aPPD and aPPT regulate CYP3A4 at the enzymatic level.

Amanda Vanden Hoek[1,2,3], Kim Talbot[1,2], Jeff Hewitt[2,4], Ross MacGillivray[2,4], Edward Pryzdial[1,2,3]

[1]Canadian Blood Services, R&D, [2]University of British Columbia/Centre for Blood Research, [3]Pathology and Laboratory Medicine Department (UBC), [4]Biochemistry and Molecular Biology Department (UBC)

Amanda Vanden Hoek

Novel function of clotting factor Xa: conversion of factor Xa into a clot-dissolving cofactor

Backround/Objectives

The purpose of the current work is to (1) delineate the mechanism of conversion of clotting factor Xa (FXa) into a clot-busting cofactor and (2) evaluate the putative role of the FXa proteolytic derivative Xa33/13 in clot dissolution. It is hypothesized that (1) FXa functional conversion is an example of a previously identified "ratcheting effect" whereby initial cleavage results in a conformational change in the molecule to present a second distal site for cleavage; (2) alteration of critical plasmin cleavage sites will impair the generation of this novel function.

Methods

Five FXa mutants were generated by single point mutation of basic residues to Gln: Lys330 in the protease domain and four residues in the beta-peptide region (Lys435, Lys433, Arg429 and Lys427). Chromogenic and clotting assays were conducted to measure recombinant FXa activity. FX activation and plasmin degradation patterns were monitored by western blotting.

Results

Preliminary findings indicate that mutation at Lys330 is sufficient to prevent generation of Xa33/13. Mutation in the beta-peptide region affects both beta-peptide excision and subsequent cleavage at Lys330. The beta-peptide region mutants will be used to elucidate the exact residue(s) involved in this first cleavage.

Conclusion

Factor Xa (FXa) is an essential blood clotting enzyme. We have previously identified a FXa proteolytic derivative, Xa33/13, which has novel function. Xa33/13 is generated by plasmin-mediated cleavage, whose normal function is to dissolve clots. Xa33/13 cannot participate in clotting, instead conferring clot-dissolving (fibrinolytic) function by acting as a tissue plasminogen activator (tPA) cofactor thereby accelerating plasmin generation. Currently, tPA is the only drug approved by the FDA for administration to myocardial infarction or stroke patients to dissolve thromboses. However, tPA can also cause severe bleeding which can be fatal. The development of a therapeutic tPA cofactor that can act only in this fibrinolytic capacity without affecting physiologic clotting (ie. Xa33/13) would reduce the occurrence of severe bleeding and make tPA a safer drug for patients with blood clots.

Abstract # 47

Chansonette Harvard

Harvard C[1], Qiao Y[1], Fawcett C[2], McGillivray B[3], Farrell S[4], Bernier F5, McLeod R[5], Hurlburt J[3], Holden JJA[6], Lewis MES[3], Rajcan-Separovic E[1]

[1]Departments of Pathology and [3]Medical Genetics, UBC, [2]Cytogenetics Laboratory, Royal Columbian Hospital, [4]Credit Valley Hospital, Mississauga, [5]Medical Genetics, University of Calgary, [6]Quenens University, Kingston

High resolution array analysis of 45 subjects with idiopathic intellectual disability (ID) using the 105K Agilent and 325K Nimblegen arrays

We have tested two commercially available high resolution arrays for their applicability in routine clinical service. The number of clinically relevant changes, number of known and new benign variants, and the methods required for confirmation and follow-up of copy number variants (CNVs) were assessed.

Selection of CNVs for confirmatory studies was based on the cut-off criteria for the two arrays*, limited overlap of detected CNVs with known variants (i.e. complete overlap with <2 variants in normal subjects) and gene content.

The Nimblegen array analysis of 15 patients detected an average of 4 CNVs per subject. Of the five changes that fulfilled the above criteria for follow-up, two were de novo duplications including 5q14.1 (9.78 Mb) and 9q21.1 (2.46 Mb), and three were novel, familial CNVs in areas not overlapping with previously reported benign variants (1q21.1, 1q44 and 7q31.1).

The Agilent array analysis of 30 subjects detected an average of 7 CNVs per subject. Of the 19 CNVs that fulfilled criteria for further follow-up, seven were tested so far by FISH, and six were confirmed. Four were de novo CNVs and included a duplication of 17q12 (1.82 Mb), and deletions of 16q22 (3.43 Mb), 2q23.1 (2.2 Mb) and 10q21.1 (1.3 Mb). The remaining two confirmed changes include a familial gain at 18p11.22 (0.9 Mb) and a CNV of yet unknown origin involving simultaneous Yp11.2 microduplication (0.18 Mb) and Yq11.223 microdeletion (0.5 Mb). The remaining 12 changes detected using the Agilent array will be followed up by FISH (10) and RT-qPCR (2).

The number of clinically relevant (de novo) changes in the two subject groups is comparable (13%) although the pick-up rate may increase for the Agilent array group when all the confirmatory studies are completed. Although approximately half of the CNVs that fulfilled the criteria for follow-up in both groups were smaller than 500kb (11/19 and 2/5 in the Agilent and Nimblegen group, respectively), the vast majority can be tested using FISH and only 4/23 will require RT-qPCR as they are very small (<100 Kb).

We conclude that the increased resolution of the Nimblegen arrays did not increase the number of CNVs requiring follow-up or the number of de novo changes. In fact, after the confirmatory studies for all CNVs detected using the Agilent array is completed, this platform may result in a higher pick-up rate of clinically relevant changes. The proportion of CNVs that can be further assessed using FISH is encouraging for a routine clinical laboratory setting.

**for both arrays at least 3 probes in a sequence should show a copy number change; for Nimblegen array log2 ratio > ±0.2 for a segmentation (region) mean is used and for Agilent the minimum absolute average log Ratio = 0.25.*

Mustafa I, Wang D and Scott MD

Canadian Blood Services and the Centre for Blood Research and the Dept of Pathology and Laboratory Medicine at the University of British Columbia, Vancouver, BC, Canada

Ibrahim Mustafa

Immunological inhibition arising from misplaced iron: implications for thalassemia and sickle cell disease

Backround/Objectives

The thalassemias and Sickle Cell Disease (SCD) arise from mutations to the globin subunits of adult hemoglobin (HbA) resulting in destabilized hemoglobin and, potentially, a life-threatening anemia due in part to iron-driven redox reactions. While transfusions corrects the anemia, secondary iron overload can occur. Thus, both the primary and secondary pathology of thalassemia and SCD arise from "misplaced" iron. Removal of oxidatively damaged RBC in vivo occurs primarily via erythrophagocytosis by the mononuclear phagocytic system (MPS). This clearance mechanism may result in negative immunoregulatory effects such as the observed increased risk bacterial infections in these patients.

Methods

To determine the functional consequences of iron on the MPS, the effects of ferric iron (Fe3+; ferric ammonium citrate, FAC), heme, purified HbA and oxidized RBC on antigen presentation/proliferation by PBMC and cultured dendritic like (DC) cells was examined. Antigens examined included tetanus toxoid (TT Ag), formalin-fixed Streptococcus mutans (SM Ag) and RhD peptide. PBMC proliferation was determined by 3H-thymidine incorporation or via flow cytometry using carboxyfluorescein diacetate, succinimidyl ester (CFSE) stained cells. To determine if iron-driven immunomodulation could be reversed, an iron shuttle chelation system using Desferal (DFO; shuttle chelator) and S-DFO (a high molecular weight DFO-starch conjugate) was examined.

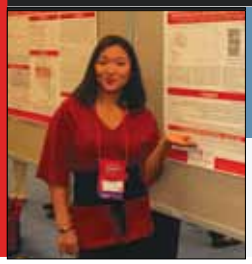
Results

Importantly, all forms of iron, including oxidized RBC, significantly inhibited antigen presentation and PBMC proliferation. For example, 100 µM hemin resulted in a >98% reduction in proliferation in response to the TT or SM Ag. Similarly, phagocytosis of oxidized RBC virtually abolished the ability of antigen presenting cells within the PBMC to present antigen and abolished the response to the TT and SM antigens. DC cells were similarly affected by FAC (200 µM) exposure (7 days) with a ~78% reduction PBMC response to an immunodominant RhD peptide. Iron chelators could partially overcome the effects of the bioreactive iron. Of interest, prolonged treatment with S-DFO (unlike DFO) did not adversely affect purified hemoglobin.

Conclusion

As shown, iron has significant immunodepressive effects on immune function (antigen presentation and lymphocyte proliferation). Iron chelation can effectively bind and remove free and complexed iron /heme preventing both redox-driven damage and immuosuppression. These data suggest that a two component iron shuttle chelation system may effectively slow/prevent iron-driven damage within cells and may also protect immune competency.

Abstract # 49



Yevgeniya Le

Yevgeniya Le[1, 2, 3] and Mark D. Scott[1, 2, 3]

[1]Canadian Blood Services, [2]Centre for Blood Research, [3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Biophysical consequences of the immunocamouflage of cells

Backround/Objectives

While ABO/RhD typing is most often sufficient for safe transfusions, significant antigenic challenges still reside on donor blood cells that can lead to clinically significant alloimmunization. To address this risk, the immunocamouflage of allogeneic cells via covalent grafting of methoxypoly(ethylene glycol) [mPEG; PEGylation] has emerged as a promising technology. Previous studies on PEGylated blood cells have demonstrated decreased immunogenicity and antigenicity while maintaining normal cell function and in vivo (murine) survival, but the biophysical mechanisms of immunocamouflage are less well understood.

Methods

Using an aliphatic amine polystyrene latex model, the importance of the physico-chemical polymer properties (length and grafting concentration) and target size (1.2 µm vs. 8.0 µm latex) on immunocamouflage was investigated. The extent of surface charge and biophysical camouflage imparted by the grafted polymer was determined using particle electrophoresis and protein adsorption analyses, respectively. Protein adsorption assays utilized fluorescently-labelled plasma proteins and quantitative fluorescent microscopy and flow cytometry.

Results

PEGylation of latex particles with succinimidyl valerate-mPEG (SVA-mPEG) resulted in surface charge camouflage that positively correlated with polymer molecular weight (MW) and grafting concentration. The mobility of 1.2 µm latex particles grafted with 2.0 mM, 20 kDa polymer decreased dramatically from 100% to 0.0±7.4% of unmodified latex. For 8 µM particles, 20 mM grafting concentration of 20 kDa SVA-mPEG decreased mobility to 31.9±2.5% of control. In contrast to surface charge, biophysical camouflage of surface-macromolecule interactions exhibited both concentration and target size dependency. It was observed that 2 kDa polymer more effectively prevented protein adsorption to small particles while the 20 kDa polymer was more effective on large particles. Furthermore, fluorescein-mPEG grafting to both sizes of particles exhibited a biphasic binding curve, with a steep initial slope corresponding to unimpeded grafting followed by a shallower slope due to steric hindrance arising from the already grafted chains.

Conclusion

The biophysical interactions of PEGylated surfaces and macromolecules involve complex mechanisms dependent on the MW and grafting concentration of the polymer as well as target cell size. Consequently, PEGylation strategies must account for the size of the cells to be modified and suggest that platelets and RBC may require different PEGylation approaches to achieve maximal immunoprotection.

Troy Clavell Sutton and Dr. Mark Scott

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

Troy Sutton

Abstract # 50

Prevention of respiratory syncytial virus infection via methoxypoly(ethylene glycol)-modification of the virus or its host cell

Respiratory Syncytial Virus (RSV) causes significant morbidity and mortality in humans. To date, no vaccines or effective treatments exist for this serious respiratory disease. Current prophylactic therapy is limited to at-risk neonates due to its high cost, and involves the administration of anti-RSV immune globulins that are ~50% effective. To attenuate or inhibit RSV infection, we hypothesized that bioengineering of either the virus particle or host cell with methoxypoly(ethylene glycol) [mPEG] would prevent viral infection. Our specific objectives were to evaluate the effects of grafting concentration, linker chemistry, polymer length, and cell polarization on viral infection and propagation.

Modification of either the virus or host cells with mPEG prevented RSV infection in a dose- and size-dependent manner. For virus modification, short chain polymers (2 kDa) were significantly more effective than long chain polymers (20 kDa). For example, plaque assays demonstrated that RSV modification with 5 mM, 2 or 20 kDa mPEG resulted in a 100 and ~82% plaque reduction, respectively. In contrast, when small polymers were used to modify the host cell they provided no protection, while long chain polymers effectively prevented infection. For example, at 48 hours post-infection at a multiplicity of infection of 0.5, grafting concentrations of 5, 7.5, and 15 mM, 20 kDa mPEG decreased infection by 45, 83, and 91%, respectively. However, grafting concentrations of the 2 kDa mPEG resulted in ~0% reduction. Importantly, with both viral and host cell PEGylation strategies, moderate to high grafting concentrations of the appropriate polymer species were able to provide near complete protection against infection in both non-polarized and polarized cells.

In conclusion, mPEG-modification of RSV or the host cell are highly effective methods for preventing viral infection. Our findings indicate that the length of grafted polymer must be matched to the size of particle targeted for modification. Consequent to the high efficacy of both PEGylation approaches, future studies should evaluate mPEG-modified RSV as a vaccine strategy, and mPEG-grafting to the nasal epithelium as a prophyla

Abstract # 51

Marissa Jitratkosol

Gerald Pfeffer[1], Michelle Mezei[1], Carmen C Li2, Marissa Jitratkosol[2], Julio S Montaner[3], Helene CF Cote[2]

[1]Division of Neurology, Department of Medicine, UBC, [2]Dept of Pathology and Laboratory Medicine, UBC [3]BC Centre for Excellence in HIV/AIDS

Mitochondrial toxicity in HIV patients manifesting as a syndrome resembling chronic progressive external ophthalmoplegia (CPEO)

Backround/Objectives

HIV and antiretrovirals can affect skeletal muscle. Although mitochondrial (mt) toxicity, muscle mtDNA depletion and myopathy have been described with HAART, mitochondrial syndromes such as CPEO have not been reported.

Methods

Three HIV-infected patients (52-58 yrs) on long time NRTI-containing HAART (10-15 yrs) were referred for neurology assessment of a CPEO-like syndrome. Chart review and in one patient (#2) for whom quadriceps and levator (eyelid) muscle biopsies were obtained, mitochondrial enzyme analysis and mtDNA investigations were performed.

Results

No patient had a family history of mitochondrial disease. All three presented with a complaint of progressive ptosis (eyelid drooping), and occasional diplopia (double vision). Examination revealed ptosis in all three and multidirectional ophthalmoparesis in two patients. Also, all patients had manifestations of HAART-induced mitochondrial toxicity, including lipodystrophy, hyperlactatemia and fatigue. One patient(#1) showed improvement of his ocular symptoms after withdrawing HAART for 3 months, one(#2) did not despite substituting T20 for ddI, one(#3) was lost to follow-up. Patient #2's levator muscle revealed mitochondrial pathology but his quadriceps biopsy, including mt enzyme analysis, was unremarkable. Muscle genetic analyses detected a rare 3.9 kb mtDNA deletion (547-4443) that has been associated with CPEO.

Conclusion

These cases suggest that combined mitochondrial toxicity from HIV infection and antiretroviral therapy can occasionally produce syndromes resembling mitochondrial disorders which may improve by withdrawing the offending agent(s). It is unclear whether HIV infection and mitotoxic agents can produce the syndrome on their own or whether they may exacerbate and unmask previously present sub-clinical mitochondrial disease. Nevertheless, the incidence of this CPEO-like syndrome, a rare disease in the general population (the prevalence of all mitochondrial diseases combined is ~1/8500), seems high considering that our total patient population on HAART is ~4500. The observation of CPEO-like syndrome may increase as the number of aging HIV survivors with long-term HAART exposure rises.

Marissa Jitratkosol, Beheroze Sattha, Helene CF Cote

Dept of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC

Marissa Jitratkosol

A mitochondrial DNA point mutation burden assay

Backround/Objectives

Point mutation accumulation within mitochondrial DNA (mtDNA) is hypothesized to contribute to aging and may also be increased by certain drugs such as nucleoside analogues used in HIV antiretroviral therapy. We developed a method to detect point mutations in the D-loop region of the human mt genome to study mtDNA point mutation burden. The assay was used on blood and muscle tissues.

Methods

For each human sample studied, total DNA was extracted and a ~462 bp region of the D-loop was amplified and cloned. From this, ~90 clones were individually sequenced and compared to the sample's consensus sequence to identify mutations. Background assay error rate was determined by subjecting an individual clone to the procedure. The effect of PCR enzyme (High-Fidelity (Hi-Fi) Taq vs. Pfu), number of amplification cycles and number of clones analysed, as well as the length of the homopolymeric cytosine (C)-tract (from C7 to C11) were investigated.

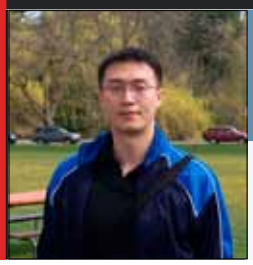
Results

At 35 cycles, Hi-Fi Taq induced ~9x more background mutations than Pfu (5.5 compared to 0.61 mutations per 10000 bp). Surprisingly, with Pfu, background error rate appears to decrease slightly with higher number of PCR cycles, that trend was not observed with Hi-Fi Taq. 98% of all mutations induced by Hi-Fi Taq were transition mutations in contrast to only 53% with Pfu. Also, with both enzymes, increased background variability in the C-tract length was seen with longer C-tracts, usually a decrease by one cytosine. With Pfu, mutation burdens ranging from 1 to 6 times background value were observed in the various tissue sample studied, with an intra-sample coefficient of variation ranging from 20-50% depending on mutation burden.

Conclusion

Based on these preliminary results, this assay can be used to detect differences in heteroplasmic mtDNA damage in clinical tissue samples and should also be suitable for human cultured cell models. Moreover,this assay should be adaptable to study other regions of the mtDNA genome and other animal models. Potential applications include the study of aging, mitochondrial disease and dysfunction, and chemotherapeutic-induced mitochondrial toxicity.

Abstract # 53



Zhen Liu

Zhen Liu, Huifang M Zhang, Lifeng Qu, Xin Ye, Decheng Yang

Cardiovascular Research Lab, The James Hogg iCapture Center-St. Paul's Hospital, Dept of Pathology and Laboratory Medicine, University of British Columbia

Interferon-gamma-inducible GTPase (IGTP) relieves ER stress response induced by coxsackievirus B3

Backround/Objectives

Endoplasmic reticulum (ER) is an important organelle for viral replication and maturation. A number of viral infections have been shown to disrupt ER function and cause ER stress. Eukaryotic cells respond to ER stress by a group of coordinated signal transduction pathways termed ER stress response, which is mediated by three ER transmembrane proteins, the PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6) and the ER transmembrane protein kinase/inositol-requiring enzyme 1 (IRE1). Evidence shows that the regulation of ER stress responses may dictate the viral replication and pathogenesis. Interferon-gamma-inducible GTPase (IGTP) is a p47 GTPase upregulated in coxsackievirus B3 (CVB3)-infected murine heart, and can inhibit CVB3-induced apoptosis.

Methods

In this study, using doxcycycline-inducible Tet-On HeLa cells that overexpress IGTP, we evaluated the effect of IGTP on ER stress induced by chemical inducers and CVB3 infection. We also confirmed the effect of IGTP on ER stress response in IGTP KO and wild type mouse embryonic fibroblast (MEF) cells by CVB3 infection and tunicamycin treatment.

Results

IGTP expression suppressed the tunicamycin- and Brefeldin A-induced upregulation of glucose-regulated protein 78 (GRP78), a master chaperon and ER stress marker. For the PERK branch of ER stress response, IGTP expression strongly inhibited the activation of PERK, phosphorylation of eIF2-alpha, and the subsequent activation of ATF4 (activating transcription factor 4). IGTP also suppressed ATF6 branch pathway by inhibiting the cleavage and nuclear translocation of ATF6, and inhibited ATF6-induced p58IPK expression as well as activation (cleavage) of sterol response element-binding protein-1 (SREBP1) simultaneously. The suppressed ER stress responses also led to the diminished phosphorylation of eIF2-alpha as well as the.reduction of proapoptotic CHOP and GADD34 expression, which was supported by the detection of decreased caspase-3 activation and cleavage of PARP. In the CVB3 infected Tet-On HeLa cells, IGTP expression also inhibited virus-induced upregulation of GRP78. The above results were further confirmed by using wild type and IGTP KO MEF cells: in wild type MEF cells the short treatment with interferon-gamma could diminish the ER stress response that was triggered by either tunicamycin or CVB3 infection; However, such relieving effect was not observed in KO MEF cells that are lacking IGTP expression under interferon-gamma treatment, suggesting that IGTP is the molecule responsible for relieving ER stress response.

Conclusion

IGTP expression relieves the ER stress responses induced by either chemical stressors or CVB3 infection, both in Tet-On HeLa cells and in mouse embryonic fibroblasts. KO of IGTP blocks the IGTP-mediated suppression of CVB3-induced ER stress. IGTP inhibits the activation of PERK and ATF6 branches of ER stress responses, and suppresses the downstream apoptotic gene expression.

Xin Ye[1], Zhen Liu[1], Mary Zhang[1], Decheng Yang[1]

[1]Dept of Pathology and Laboratory Medicine, University of British Columbia, The Heart and Lung Institute, St. Paul's Hospital, Vancouver, Canada

Xin Ye

An artificial microRNA targeting the 3'UTR of coxsakievirus B3 inhibits viral replication

Backround/Objectives

Coxsakievirus B3 (CVB3) is the leading pathogen causing viral myocarditis. CVB3 has a positive single-stranded RNA genome which encodes a single open reading frame flanked by the 5' and 3' UTRs (untranslated regions). The 3'UTR is 99 nts long with a secondary structure including three stem loops. The interactions among these stem loops are extremely essential for the viral replication. In addition, siRNAs targeting the 3'UTR of CVB3 were demonstrated to inhibit the viral infection.

MicroRNAs (miRNAs) are a family of endogenous short non-coding RNAs with post-transcriptional regulatory functions. They are capable of mediating the gene silencing by targeting the 3'UTR of mRNAs. Since miRNA expression profiles were found to be associated with various diseases such as cancer, cardiovascular disease and viral infection, their potential in therapeutic application is under investigation. One of the novel methods is artificial miRNA (amiRNA) mediated gene silencing. amiRNAs are designed according to the characteristics of natural miRNAs, including:

i) 5'end seeding sequence of miRNA should be complementary to the target sequence;

ii) 3' end complementary sequence enhances the targeting efficiency;

iii) The target site should be close but not too close to the 5' end or 3' end of the 3'UTR sequence;

iv) Several mismatches in the middle of the miRNA may be beneficial.

Objective: We are aiming to design artificial miRNAs to inhibit the CVB3 infection by targeting the 3'UTR of CVB3 genome.

Methods

amiRNAs targeting the 3'UTR of CVB3 genome are designed according to the basic characteristics described above. Considering the importance of loop structure in CVB3 replication and the energy requirement by possible interaction between miRNA and the target sequence, three miRNA candidates were designed to target the middle loop domain since it is not too close to the 5' end or 3' end of the sequence. Moreover, this domain has the biggest loop structure which may be easier for miRNA binding. Natural miRNAs are maturated from their precursor form --pre-miRNAs. To mimic this process, the designed amiRNAs were incorporated to in vivo pre-miRNA hsa-mir-126. Stable HeLa cell lines expressing these pre-amiRNAs were established and the antiviral activity was evaluated by microscopy analysis of cell morphology, Western Blotting to detect viral protein and viral plaque assay to measure viral titer.

Results

Compared with other candidates, amiR1 cell lines showed significantly reduced cell death after infection of CVB3. The viral protein VP1 expression level was much lower in amiR1 cell lines than other ones. Viral plaque assay also proved the downregulated viral replication due to the amiR1 expression.

Conclusion

Preliminary data suggest that amiR targeting the 3'UTR of CVB3 genome can inhibit CVB3 replication. amiR1 is the most promising candidate among the amiRNAs tested. Further studies will be carried out to confirm this effect and apply this amiRNA in cardiomyocyte cell lines and *in vivo* studies.

Abstract # 55

Johnny Chuanyi Nie

Chuanyi Nie, Brian Chung, Dong Jun Zheng, Rusung Tan and Peter van den Elzen

Department of Pathology & Laboratory Medicine, University of British Columbia & BC Children's Hospital, Vancouver, Canada

CD1d-restricted endogenous lipid antigen presentation during EBV infection

Backround/Objectives

Natural killer T (NKT) cells belong to a unique lymphocyte population characterized by their reactivity against glycolipids bound by a non-classical major histocompatibility complex (MHC) molecule, CD1d. Recognition of self or foreign-lipid/CD1d complexes rapidly induce NKT cells to secrete the immunomodulatory cytokines and become cytotoxic against CD1d-positive targets. The immediate response of NKT cells is thought to initiate and direct subsequent adaptive immune responses against a wide range of pathogens. Studies have shown that viruses have acquired means to interfere with lipid antigen presentation by disrupting CD1d trafficking. The existence of mechanisms that interfere with CD1d-mediated lipid presentation implies a role for NKT cells in the detection and elimination of infected cells.

Preliminary experiments showed downregulation of CD1d molecules in B cells infected with the human herpesvirus Epstein-Barr virus (EBV). We hypothesize that B cells upregulate antigenic self-lipids that stimulate NKT cells upon EBV infection. This study addresses how NKT cells are activated during viral infections and may lead to novel therapeutics targeting lipid antigen presentation and NKT cell activation.

Methods

Tonsillar B cells isolated from healthy individuals were infected with EBV 95.8 and treated with or without AM580, a compound that artificially upregulates the surface expression of CD1d. Untreated or AM580-treated infected B cells were co-cultured with NKT cells in the presence or absence of *alpha*-galactosylceramide (*alpha*GalCer), a NKT cell-specific agonist. Anti-CD1d antibody treatment was used as control for non-specific CD1d activation of NKT cells. CD1d upregulation was measured by flow cytometry and the NKT cell activation was measured using ELISA for IFN-y production.

Results

Infected B cells treated with AM580 and loaded with *alpha*GalCer stimulated significantly more NKT cell IFN-*y* production compared to untreated *alpha*GalCer-loaded infected B cells. Interestingly, NKT cells also produced IFN-gamma when cultured with AM580-treated B cells in the absence of *alpha*GalCer. This observation suggests that infected B cells upregulate endogenous lipid antigens following EBV infection.

Conclusion

AM580 augmented the ability of EBV-infected tonsillar B cells to stimulate NKT cell activation. Although further confirmation is needed, our preliminary data suggests that EBV infection of B cells increases endogenous lipid antigen presentation. These results suggest that AM580 treatment may a therapeutic candidate for treatment of EBV-related illnesses.

Audra Vair, Sharlene Eivemark, Hermann Ziltner, Richard Stokes

Child and Family Research Institute, UBC

Audra Vair

CD43 signalling in macrophages in response to Mycobacterium tuberculosis infection

Backround/Objectives

Despite the availability of a vaccine and therapeutics, Mycobacterium tuberculosis (M.tb) remains an extremely important global pathogen. More than 1.5 million people die and 8 million new infections are reported annually. Understanding the process of infection and persistence of this intracellular pathogen will facilitate the discovery of mycobacteria-specific methods of treatment and prevention.

CD43 is a highly glycosylated transmembrane protein that is expressed on the surface of most hemapoietic cells, including T lymphocytes, plasma cells, neutrophils, platelets and mononuclear phagocytes. Its function has been well-characterized in many of these cells, but CD43 macrophage-related functions are not yet well-defined.

Previously, our lab observed differential levels of binding and intracellular replication of M.tb in CD43 knockout and wild-type murine macrophages. The absence of CD43 on the macrophage resulted in a decrease in binding, but increased intracellular replication and severity of infection (Randhawa et al, 2005). Further investigation uncovered differential expression of IL-12, IL-6 and TNF-alpha between the CD43 +/+ and -/- macrophages as well as sub-optimal levels of apoptosis and increased levels of necrosis in the CD43 -/- macrophages (Randhawa et al, 2008). The objective of this study is to investigate the intracellular pathways that are involved in response to the ligation of CD43 by whole bacteria and by proteins identified as bacterial ligands for CD43.

Methods

CD43 -/- and +/+ murine macrophages were cultured in vitro and exposed to (one of) : Phosphate-buffered Saline (control) , recombinant M.tb dnaK protein, recombinant M.tb Cpn 60.2 protein, or Mycobacterium tuberculosis (whole, live bacteria). RNA was isolated, mRNA was amplified, labelled and applied onto RNA Microarrays. Data was analyzed using Genespring and Ingenuity Pathway Analysis. Further qualification (RT-PCR, Western Blot) of results with follow.

Results

Not surprisingly, there was a greater quantity of differentially-expressed genes between CD43 -/- and CD43+/+ macrophages in response to M.tb exposure than either of the two recombinant proteins. Interestingly, few of these genes were shared between all three groups. The greatest similarities in gene expression were between the protein-whole bacteria comparisons, as opposed to protein-protein comparisons. A number of intriguing pathways appear to be impacted by ligation of CD43 including the differential expression of genes involved in apoptosis, lipid metabolism (prostaglandin and leukotriene sythesis) and cytoskeletal remodelling.

Conclusion

The binding of M.tb by macrophage results in a transcriptome that is affected by the presence or absence of CD43. Ligation of this receptor with individual proteins known to interact with CD43 results in subsets of expression specific to each protein and to be involved in cellular pathways connected to mycobacterial survival within the macrophage. Qualification of differential mRNA and protein expression will follow.

Abstract # 57

Billie Velapatino

Billie Velapatiño[1], Sharon Peackok[2], Narisara Chatratita[2], and David P. Speert[3]

[1]Dept of Pathology and Laboratory Medicine, UBC, Vancouver, British Columbia, Canada, [2]Mahidol University, Bangkok, Thailand, [3]Division of Infectious and Immunological Diseases, Dept of Pediatrics, UBC and Centre for Understanding and Preventing Infections in Children, Vancouver, British Columbia, Canada.

Analysis of the potential mechanisms of latency in burkholderia pseudomallei, the most common cause of fatal bacterial infection in regions of Southeast Asia

Backround/Objectives

Burkholderia pseudomallei (*BP*) is an aerobic, Gram-negative bacterial organism and is the causative agent meliodosis. Meliodosis is characterized by acute pneumonia and septicaemia in tropical regions of Southeast Asia and Northern Australia, mostly in immunocompromised individuals. At present there is no available vaccine or effective treatment for this disease. Reports showing multiple cases of Meliodosis in veterans after the Vietnam conflict as well as in returning travelers from endemic regions, suggest that *BP* could remain dormant in patients for prolonged periods and this dormancy could be linked to colony variation (morphotypes I-VI). Therefore, just like with tuberculosis, latency with reactivation decades after infection can also occur.

Objectives: To identify proteins of BP that may be crucial during periods of persistence and to examine whether the BP proteome changes over time *in vivo*

Experimental approach

Total proteins from BP strains isolated from patients who have relapsed after treatment within a year were purified. Up-regulated or down-regulated proteins from the primary and from the relapse strains were identified by two-dimensional (2D) gel electrophoresis (2D-PAGE). Protein spots were then excised from 2D-PAGE gels and were analyzed using Mass Spectrometry at the Genome BC Proteomics Centre, University of Victoria, BC.

Results/Conclusions

10 differentially expressed proteins were identified in one patient. 3 proteins were present in the primary isolate (morphotype I) but were absent or down-regulated in the relapse strains (morphotype I and III); including a putative Hemolysin-coregulated Protein (Hcp1). This protein may be a member of the T6SS virulence cluster genes. 7 proteins were up-regulated in the relapse strain, including flagellin and Hsp20 alpha crystalline family proteins; which are present in response to stress conditions; the latter is one of the major immunoreactive proteins of latent *M. tuberculosis*, suggesting that the bacteria may possibly adopt this morphology (III) to survive inside the host. Appreciation of the factors that affect latency and activation of Meliodosis will require clarification of the mechanisms by which *BP* shifts down to the dormant state and back up to active growth.

Farshid S. Garmaroudi[1], David Marchant, Ali Beshashati, Abbas Khalili, Mitra Esfandiarei, Raymond T. Ng, Kevin Murphy, Honglin Luo and Bruce M. McManus

The James Hogg iCAPTURE Centre, Heart + Lung Research Institute, Dept of Pathology and Laboratory Medicine

Farshid S. Garmaroudi

Abstract # 58

Systems properties of signaling components: IkappaBalpha is a hub signaling molecule in virus-infected cardiomyocytes

Backround/Objectives

Viruses must extensively manipulate host cell machinery to support viral replication. Meanwhile, infected cells mobilize an array of defence mechanisms to battle the invader. We and others have already shown the role of distinct signaling pathways (local properties) to support coxsackievirus B3 (CVB3) replication. We propose that cell signaling pathways are not autonomous units, but conjoined networks (global properties). Despite the key roles already shown for signaling networks in determining cellular function during infectious encounters, their global structure remains elusive. Overlapping functions of signaling components occurs in CVB3-infected cardiomyocytes, enabling CVB3 to progress through its life cycle.

Methods

To study the global properties of signaling networks, we generated a 3-dimensional data set: 1) signaling and viral components: nine signaling molecules and two virus replication indicators; 2) 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 3) 23 different experimental conditions. We utilized graphical Gaussian modeling (GGM), Bayesian model averaging (BMA), and clustering to verify crosstalk between and among signaling pathways.

Results

We show that: 1) p38 MAPK (p38 mitogen-activated protein kinase) provokes crosstalk with other pathways at 8 h p.i.; 2) IkBa (inhibitor of NFkB alpha protein) plays a hub signaling role in assembling this network structure, and 3) CVB3 protein synthesis (VP1 expression) is negatively correlated with phosphorylated ATF-2 (activating transcription factor).

Conclusion

Constructing a systems-level and data-driven model allows us to generate new knowledge of how CVB3 evolved multiple strategies to regulate intracellular signaling networks in order to sustain its replication. Understanding the mechanisms that explain functional properties of signaling networks throughout infection will provide breakthroughs in the exploring of the master regulators that can be used as therapeutic targets for pharmacological interventions in the treatment of viral myocarditis and its major sequela, dilated cardiomyopathy.

Abstract # 59

Yu-Hsuan Huang

Yu-Hsuan Huang, John Priatel, and Rusung Tan

Child & Family Research Institute BC Children's Hospital and Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

The role of SAP in the differentiation of IL-17 producing T cells

Backround/Objectives

Epstein Barr virus (EBV) is a ubiquitous herpesvirus that can cause severe human disease in immunosuppressed individuals. X-linked lymphoproliferative syndrome (XLP) is a rare, inherited immunological disorder that is characterized by acute lymphoproliferation following infection with EBV, resulting in loss of viral control and often fatal complications. About 50% of XLP patients have mutations in a small protein called SLAM-associated protein (SAP). SAP is a small signaling adaptor molecule that likely plays critical roles in both the innate and adaptive immune systems since it is expressed in both natural killer (NK) cells and T cells. The justification for the study of SAP function is not only for the benefit of boys born with XLP but also for understanding how the body orchestrates the complex immune reactions against EBV.

Our lab as well as others have previously described that SAP is required for the development of natural killer T (NKT) cells in both mice and humans. NKT cells as well as other innate T cell lineages like gamma delta T cells have been implicated in the production of the cytokine IL-17. IL-17 is thought to stimulate production of inflammatory cytokines and chemokines facilitating the recruitment of macrophages and neutrophils, mediate protective immunity to pathogens, such as Salmonella, Mycobacterium and Listeria and has been implicated in the pathogenesis of organ-specific autoimmune diseases. Notably, we have found that SAP-deficient mice are highly susceptible to Listeria monocytogenes (LM) infection. The mechanism regulating the differentiation of naïve T cells into IL-17 secreting effectors are poorly understood. Therefore, we hypothesize that SAP is involved in the differentiation and/or function of IL-17 producing T cells and the shortage of such T cell lineages result in immunodeficiency against some microbial infections.

Methods

To address these questions, we have utilized wild type and SAP deficient mice and compared the frequencies of IL-17 positive T cells after in vitro stimulation. Briefly, splenocytes from wild type and SAP-deficient mice are stimulated with anti-CD3, anti-CD28 antibody and cytokines. Subsequently, cells are re-stimulated with PMA/Ionomycin to mediate detection of intracellular staining of IL-17 by flow cytometry.

Results

In wild type mice, the frequency of CD4+ IL-17-producing cells is seven times as compared to SAP mutant. Furthermore, we also found that there is two- to three-fold increases in IL-17 producing CD8+ gammadelta T cell positive populations in SAP WT mouse.

Conclusion

Here, we report that SAP positively regulates the differentiation of naïve T cells into IL-17 producing effectors. Further studies will be designed to study the relationship between SAP and IL-17 producing T cells in protective immunity following infection of wild type and SAP-deficient mice with LM. The signaling pathway of SAP in regulating IL-17 production will also be analyzed.

Lenka Allan, Katrin Hofl, Dong-Jun Zheng, Brian Chung, Rusung Tan, and Peter van den Elzen

Department of Pathology, Child and Family Research Institute, University of British Columbia, Vancouver, BC, Canada

Lenka Allan

Apolipoprotein-mediated lipid antigen presentation in B cells

Backround/Objectives

B cells that specifically internalize antigens via the BCR-mediated pathway are 100-1000 times more efficient at stimulating T cells than other professional APCs when antigen concentration is limiting. However, recent studies suggest that B cells may also be capable of presenting internalized lipid antigens to Natural Killer T (NKT) cells by non-conventional mechanisms that are independent of the BCR. NKT cells are innate-like T cells that have been shown to induce proliferation and enhance immunoglobulin production in B cells in a CD1d-dependent manner. However, the mechanisms by which B cells internalize lipid antigens by non-BCR-mediated pathway have not been explored. In previous work, we have shown that LDL-receptor-mediated uptake of apolipoprotein E (ApoE)-bound lipid antigens by dendritic cells (DCs) can effectively promote NKT activation. Therefore, we explored pathways by which B cells may internalize lipid antigens in a non-BCR mediated mechanism, focusing first on uptake of ApoE-bound lipid antigen.

Methods

Cell culture (NKT cell assays measuring interferon gamma production by ELISA), Proliferation and activation determination (FACS based), Immunoglobulin production (B cell IgG secretion measured by ELISA.)

Results

Here we report that human B cells, like DCs, present Apo-E bound lipid antigens more efficiently to NKT cells, than they do free lipid antigen. ApoE-delivered lipid antigen dramatically enhanced NKT activation. The enhanced NKT activation was observed with ApoE3 and ApoE4 but not with ApoE2, a variant defective in LDL-R binding. Furthermore, the enhanced NKT activation was blocked with LDL-R antibodies. These findings indicate that ApoE-bound lipid uptake requires the LDL-R. Interestingly, we also found that activated B cells up-regulate LDL-R expression and are more efficient at presenting ApoE-delivered lipid to NKT cells, than are resting B cells.

Conclusion

Therefore, we propose that B cells that internalize ApoE-bound lipid antigens through LDL-R-mediated endocytosis can illicit activation of T cells with which they that do not share antigen specificity. This non-BCR-mediated pathway of antigen presentation is a form of non-specific cognate NKT help.

Abstract # 61

Nicholas Rossi

Nicholas A.A. Rossi[1,2], Iren Constantinescu[1,2], Donald E. Brooks[1,3], Mark D. Scott[1,2], Jayachandran N. Kizhakkedathu[1]*

[1]Centre for Blood Research, Dept of Pathology and Laboratory Medicine, [2]Canadian Blood Services, and [3]Department of Chemistry, University of British Columbia

A highly efficient and novel cell surface derivatization technology based on diffusion-modulated macromolecular transport and reactivity

Backround/Objectives

To improve in vivo compatibilities, considerable effort in the area of biomedical research has centered on the covalent or non-covalent grafting of non-toxic polymers to proteins, peptides, cell surfaces, drugs, and implants. Polymeric cell derivatization technology has developed from the need to prolong circulation times, increase resistance to enzymatic degradation, and minimize immunogenic responses of certain cells in the body. In particular, our group has been investigating the derivatization of red blood cells, with the expressed aim of camouflaging surface antigens to develop a universal red blood cell. Often, excess polymer is required since derivatization is inefficient; this is costly and can cause side reactions and increased toxicity (e.g. from the reactive groups situated on the polymers). Here, a novel and highly efficient Diffusion-Modulated Macromolecular Cell Derivatization (DMMCD) technology in which non-reactive `additive' polymers are used to significantly enhance cell surface derivatization with reactive (functionalized) polymers is described.

Methods

Since the reactive polymers also contain fluorescent labels, the extent with which reactive polymers were covalently grafted to four different cell types – namely red blood cells, white blood cells, platelets, and T-cells – was followed using flow cytometry. The cell compatibility of the process of using 'additive' polymers to enhance derivatization was followed using optical microscopy, cell counting, and size distributions. The extent with which red blood cells were antigenically camouflaged (RhD) was determined using FITC-labeled Anti-D antibodies and flow cytometry.

Results

The presence of an additive polymer (either hyperbranched polyglycerol or dextran) in increasing amounts led to enhanced grafting of two types of fluorescein-labeled, succinimide functionalized polymers – linear poly(ethylene glycol) and hyperbranched polyglycerol. Depending on the type or amount of additive polymer, a two to ten fold increase in the amount of covalently bound polymer was routinely observed. The importance of this demonstration of enhanced efficiency and the possible mechanisms involved are discussed. To highlight the practical applications of DMMCD, red blood cell surface antigen camouflage was significantly improved when additive polymer was used to enhance reactive polymer grafting.

Conclusion

A novel and universal strategy for significantly enhancing macromolecular cell surface modification based on increased diffusion of macromolecules and the enhanced activity of proteins in concentrated polymer solutions is described. The universality of the technique suggests that it can be applied to other macromolecular systems and cell types. DMMCD could potentially be applied to various live cell based biotechnologies and could be used to enhance the concentration of macromolecular agents on cell surfaces.

Moshe Mishaeli[1], Abd Alnaser Zayed[1], Rinat Yerushalmi[2] , Tom Thomson[3], David Huntsman[1], Karen Gelmon[2]

[1]BC Cancer Agency - CTAG lab , [2]BC Cancer Agency - Oncology department, [3]BC Cancer Agency - Laboratory medicine

Moshe Mishaeli

Her-2 expression in circulating tumor cells and in the primary tumor of breast cancer patients

Backround/Objectives

Metastatic spread is the main cause of cancer-related morbidity and mortality in breast cancer patients. It is believed that the shedding of cells from the primary tumour into the blood circulation is an important mechanism in the spread of the disease. These cells are called Circulating tumor cells (CTCs). The number of CTCs before, during and after treatment is an independent prognostic factor in patients with metastatic disease. The Her-2 status of the primary tumor is important prognostic and predictive factors and necessary for treatment recommendations. Comparisons between the HER-2 status of the primary tumour and the metastases have revealed a wide range of discordance (7-37%) However there is no clear data describing the correlation of the Her-2 status between the primary tumour and the CTCs in metastatic breast cancer.

Methods

CTCs Enrichment procedure - 7-10 ml of peripheral blood was mixed with a RBC lysis buffer. Cells were centrifuged at room temperature and cell pellet was resuspended. Cell enrichment reagents including anti-CK and anti-EpCAM magnetic beads were added. After incubation, cells were centrifuged and subsequently applied to a magnetic column (OctoMACS). Enriched circulating tumor cells were eluted and directly deposited onto PLL glass slides by cytospin. Cells were fixed with acetone and stained with a FITC-conjugated anti-CK mix (anti-CK 8, 18 and 19) and DyLight 549-conjugated anti-CD45; nucleus was stained with DAPI. In addition, cells were also stained with a modified hematoxylin brightfield dye for morphological information.

Image analysis - CTC samples were evaluated with the Ariol system. Slides were scanned, automatically imaging cells in three fluorescent channels and brightfield. To be classified as a circulating tumor cell the cell should be positive for CK and for nuclear staining and negative for CD45. In addition, the brightfield imaging shows cell morphology, allowing discrimination between cells and artifacts like cell debris or cell fragments. When over 5 CTCs were detected, a Her-2 FISH was performed on the same slide.

DNA FISH Analsyses - The PathVysis Her-2 DNA probe kit (Abbott Laboratories, USA) was used to label the CTC (SpectrumGreen for CEP 17, and SpectrumOrange for Her-2). The probe was applied to the CTC slides, denatured at 73˚C and then incubated at 37˚C overnight. A second , fast CTC-targeted was done. The images for CTC identification and FISH analyses were fused to obtain and score the FISH status on the specific tumor cells. The results were compared to the Her-2 status of the primary tumour.

Results

84 samples were scanned and in 11 cases a Her-2 FISH was done on the CTCs. In all of the cases the Her-2 status was identical on both of the samples - the primary tumor and the CTCs.34.5% and 78.8% of cases, respectively. Ki-67 expression was very low (1-5%) and did not distinguish between the atypical and non-atypical CCLs.

Conclusion

Early results of comparing Her-2 status on primary breast tumor and circulating tumor cell in the same patient show a high concordance. CTCs could potentially be used as a platform to assess the Her-2 status of metastatic breast cancers when the primary tumour is unavailable or temporally remote.

Abstract # 63

Katherine Serrano

Anna Prudova[1], Ulrich auf dem Keller[1], Katherine Serrano[1,2], Dana V. Devine[1,2], Christopher M. Overall[1]

[1]Centre for Blood Research, UBC, [2]Pathology and Laboratory Medicine, UBC

Characterization of proteolytic processing in stored platelets using terminal amine isotopic labeling of substrates (TAILS)

Backround/Objectives

Platelets are a critical component of blood that aid in blood clotting. Donor-derived platelet concentrates are most commonly used to support burn victims and patients with severe bleeding conditions. During storage, platelets undergo poorly characterized changes defined as the platelet storage lesion (PSL). PSL is detrimental to platelets posttransfusion clotting function and limit their storage time to 5-7 days. Literature suggests that proteases play a major role in PSL initiation and progression. Our study investigates the molecular mechanisms of protease involvement in PSL.

Methods

We have designed a novel proteomic screen where the samples are isotopically labeled at the protein level (allowing characterization of proteolysis-derived neo-N-termini and proteins original N-termini). This screen, termed TAILS (Terminal Amine Isotopic Labeling of Substrates), was employed to characterize platelets stored for 9 days with or without protease inhibitor cocktail.

Results

We have identified 759 peptides corresponding to 466 proteins (compared to 1160 proteins without N-terminal enrichment). These peptides include 401 original N-termini and 358 proteolysis-derived peptides. Protease inhibitor incubation caused a >20% decrease in the amounts of 121 peptides. Further, specific protease inhibitors are being used to connect each protease with its own subset of substrates.

Conclusion

Proteases cleave specific platelet proteins during platelet storage. This may contribute to the platelet storage lesion.

Gulisa Turashvili[1], Malcolm Hayes[2], Shahriar Dabiri[2], Blake Gilks[3], Fang Lu[3], Ozge Goktepe[1], Steven McKinney[1], Peter Watson[2], Samuel Aparicio[1]

[1]Molecular Oncology and Breast Cancer Program, BC Cancer Research Centre, Vancouver, [2]Department of Pathology, BC Cancer Agency, Vancouver, [3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver

Gulisa Turashvili

Immunophenotypic analysis of columnar cell lesions of the breast

Backround/Objectives

Columnar cell lesions (CCLs) are common abnormalities in the adult female breast, characterized by the presence of columnar epithelial cells in enlarged terminal duct lobular units. CCLs are being seen increasingly in core biopsies taken for the non-palpable calcifications in screening mammography programs, and they may represent the earliest histologically identifiable, non-obligate precursor of low-grade breast cancer. The objective of this study was to determine the immunohistochemical (IHC) phenotype of CCLs.

Methods

A tissue microarray was constructed from 151 CCLs including 49 cases (32.5%) of columnar cell change (CCC) without atypia, 15 cases (9.9%) of CCC with atypia, 47 cases (31.1%) of columnar cell hyperplasia(CCH) without atypia, and 40 cases (26.5%) of CCH with atypia. IHC was performed using a series of biomarkers: estrogen and progesterone receptors (ER, PR), Bcl-2, Ki-67, cyclin D1, p53, epidermal growth factor receptor (EGFR), cytokeratin 5/6, cytokeratin 18, lactofferin, transforming growth factor, beta receptor II (TGFBR2), and somatostatin receptor 2 (SSTR2). For each biomarker, the percentage of positive cells and staining intensity were evaluated. The associations between the four different types of CCLs and biomarkers were investigated. The statistical significance was determined by two-sided chi-square tests (maximum likelihood ratio). SPSS 16.0 was used for statistical computations.

Results

Based on the exploratory analysis, CK5/6 was variably expressed in both basal (39.1%) and luminal (48.1%) cells, and the percentage of CK5/6+ luminal cells was higher in CCLs with atypia (p=0.029). Luminal cells in CCLs without atypia tended to be CK5/6 negative (p=0.017). When CCL subtypes were binarized into `CCLs with atypia' and `CCLs without atypia', lower expression of cyclin D1 (65.9%) (p=0.042) was observed in CCLs without atypia. P53 was weakly expressed in 5-10% of cells in 91.6% of cases, with higher expression in CCLs with atypia (p=0.015). No association was observed between CCL subtypes and ER, PR, Bcl-2, lactofferin, TGFBR2 or SSTR2. Calponin and EGFR were expressed in myoepithelial cells in 100% and 63.6% of cases, respectively. ER, CK18, SSTR2 and PTEN were expressed in luminal cells in all cases. PR, Bcl-2, lactofferin and TGFBR2 were expressed in 84.3%, 86.3%, 34.5% and 78.8% of cases, respectively. Ki-67 expression was very low (1-5%) and did not distinguish between the atypical and non-atypical CCLs.

Conclusion

These exploratory findings are suggestive of mature luminal cell differentiation of the different subtypes of CCLs. Since the differentiation and function of the epithelium of the normal mammary gland depend on steroid and other hormones mediated by the cellular expression of receptors, it is likely that the development of CCLs depends on stimulation of the mammary epithelium by steroid hormones and/or growth factors.

Abstract # 65

Mary Zhang

Mary Zhang, Xin Ye, Yue Su, Zhen Liu and Decheng Yang

Department of Pathology and Laboratory Medicine, University of British Columbia, The Heart and Lung Institute, St. Paul's Hospital, Vancouver, Canada

Coxsackievirus infection activates ATF6a/IRE1-XBP1 pathways and induces ER stress mediated apoptosis through $p58^{IPK}$ suppression and CHOP and SREBP1 activation

Backround/Objectives

Coxsackievirus B3 (CVB3) is a RNA virus and the most common pathogen of viral myocarditis, particularly in children and adolescent. Our previous studies and others have demonstrated that CVB3 infection induces cardiomyocyte apoptosis, leading to damage of myocardium. However, the molecular pathogenesis of the disease remains unclear, especially the CVB-activated signaling pathway that initiates the apoptotic program has not been identified.

Methods

In this study, by using cardiomyocytes and Tet-On inducible HeLa cells, we focused our investigations on the induction of the endoplasmic reticulum (ER) stress by CVB3 infection and the activation of three branches of ER stress response pathways. The major techniques used include the application of siRNAs targeting specific genes to down-regulate the signaling gene expression, Western blotting to detect the expression or phosphorylation of a number of target genes, MTT assay to determine the cell viabilities, and plaque assay to determine the virus titre.

Results

We found that CVB3 infection triggered ER stress and differentially regulated the three arms of unfolded protein response (UPR) pathways initiated by the proximal ER stress sensors: the ATF6a (activating transcription factor 6a), IRE1/XBP1 (X box binding protein 1) and PERK (PKR-like ER protein kinase). We first demonstrated the up-regulation of glucose regulated protein 78 upon CVB3 infection. We also showed that ATF6a was activated by cleavage during CVB3 infection and in turn activated XBP1 through mRNA splicing. This activation cascade was further evidenced by its up-regulation of the UPR target genes ERdj4 and EDEM1and further this target gene up-regulation could be blocked specifically by siRNAs targeting XBP1. Surprisingly, another target gene p58IPK, which is usually up-regulated in many other systems, was down-regulated at both mRNA and protein levels after CVB3 infection. These findings were further solidified by using Tet-On inducible HeLa cells over-expressing the ATF6a or XBP1. In searching for the cross-talk among the three arms of UPR pathways we found that $p58^{IPK}$, a negative regulator of PERK and PKR, may serve the linkage between ATF6a/IRE1-XBP1 and PERK because ATF6a-induced down-regulation of p58IPK benefited activation of PERK (or PKR) and phosphorylation of eIF2*alpha*. Finally, we also found the induction/activation of pro-apoptoic transcription factors CHOP (c/EBP homologous protein) and SREBP1 (sterol regulatory element binding protein 1) and activation of caspase-12.

Conclusion

Our data suggest that CVB3 infection activates UPR pathways and induces ER stress-mediated apoptosis through suppression of P58IPK and induction/activation of CHOP, SREBP1 and caspase-12.

Tracy Chan[1,2], Annie Mak[2], Stephanie Man[2], Danuta Skowronski[2], Mel Krajden[1,2], Martin Petric[1,2]

[1]UBC Department of Pathology and Laboratory Medicine, [2]BC Centre for Disease Control

Tracy Chan

Abstract # 66

Detection of influenza a virus resistance to oseltamivir by a single nucleotide polymorphism-based assay

Backround/Objectives

The H1N1 subtype of influenza A virus has recently been recognized to manifest increasing prevalence of the H274Y mutation in its neuraminidase gene (NA) that is associated with resistance to Oseltamivir. An assay capable of rapidly detecting this mutation is essential for the appropriate utilization of Oseltamivir.

Methods

An assay to detect a single nucleotide polymorphism (SNP) in influenza virus in a clinical specimen was developed based on hybridization with 2 probes; one containing the wild type nucleotide and labeled with VIC and the second containing the mutant nucleotide and labeled with FAM. Nucleic acid from clinical samples was extracted using the BioMerieux EasyMag. Samples were tested by real time RT-PCR and real time SNP assays on an ABI 7900. The SNP assays were performed using the Invitrogen Superscript III RT and the Platinum Taq polymerase, as a one-step or two-step reactions. A second two-step reaction was carried out using the proprietory ABI polymerase. An aliquot was also subjected to RT-PCR using the Qiagen 1-step RT-PCR reagent followed by cycle sequencing on an ABI 3100.

Results

A total of 141 respiratory specimens, received between Nov 14, 2008 and Mar 10, 2009, were found to be positive for the influenza A/H1 virus by RT-PCR. Of these, the first 30 specimens had undergone parallel assays to compare the reliability of a one-step and two-steps RT-PCR SNP assays. Our results indicated that 29 specimens have the resistant mutation (H274Y) by the two-step SNP assay, and the one-step assay detected the resistant mutation in 26 specimens. When tested with the ABI polymerase, only 24 were detected. Of the 29 specimens having the resistant virus by the two-step SNP, 28 were confirmed by sequence analysis of the NA gene. Subsequently, the remaining 111 specimens were tested by the one-step SNP assay because of its efficiency and easier handling. If a conclusive result was not obtained, the specimen was retested by the two-step assay. Of the 141 influenza A/H1 specimens, 128 were found to have the resistant mutation, 12 did not have sufficient amount of RNA, and 1 carried both the wildtype (H274) and the resistant (H274Y) strain.

Conclusion

The SNP assay provides a rapid, next-day turn around for the assessment of the influenza A/H1N1 virus H274Y mutation which is associated with Oseltamivir Resistance. This assay provides timely and critical results for appropriate patient management.

Abstract # 67

Sara Giesz

Sara C Giesz, Rebecca O Barnes, Janet E Wilson-McManus, Ian R Mackenzie, David G Huntsman, Michael M Burgess, Anne Junker, Blake Gilks, Sam Aparicio, Erik Skarsgard, Bruce M. McManus[1], Peter H Watson

University of British Columbia,Vancouver, BC Canada

Practical solutions in biobank facilitation: the BC biolibrary initiative

Backround/Objectives

Biobanking focuses on accrual and annotation of biospecimens, but equally critical is the creation of processes for engaging the public before accrual, distributing biospecimens, and cultivating inter-biobank collaborations. Additional focus on development of synergy between public and biobanks and these processes will enhance scientific and technological advancement and the translation of discovery to the clinic.

Methods

The BC BioLibrary is a novel, province-wide strategy aimed at public engagement in biobanking, facilitating biospecimen and data acquisition and integration of existing biobanks and research facilities into a functional and accessible framework. Built on evolutionary concepts including repatriation of biospecimen collection back into pathology departments (Biospecimen Collection Units -BCU) the BioLibrary has created a common framework governed and shared by all biobanks independent of institution or health research focus.

Results

The BC BioLibrary is embraced by leaders across BC (population 4.4M) spanning 4 hospital sites, 2 health authorities, 3 funding agencies, and major academic institutions and translational research groups. The first BCU was established in 2008 and has collected >500 biospecimens. This pilot BCU has been used to develop 18 protocols and ethics board approval for a provincial model. Two of 10 additional planned BCUs will become operational in 2009, expanding the BioLibrary into a network.

Conclusion

The BC BioLibrary facilitates access to consented, high quality, annotated biospecimens for biobanks and researchers. By providing a common infrastructure, the model increases efficiency and connection between biobanks and providers, offers a transparent process for donors and enhances public trust in biobanking.

David Marchant[1], Caroline Cheung[1], Samuel Wadsworth[1], Jon Carthy[1], Julie Ng[1], Zongshu Luo[1], Richard G. Hegele[1], Christopher M. Overall[2], Honglin Luo[1] and Bruce M. McManus[1]

[1]The James Hogg iCAPTURE Centre, [2]The Dept of Oral Biological & Medical Sciences, UBC

David Marchant

Matrix metalloproteinase-12 regulates innate anti-viral immunity via interferon-alpha

Matrix Metalloproteinase (MMP)-12 activity has been attributed to cleavage of cytokines, and digestion of extracellular matrix components related to the pathogenesis of diverse conditions, including atherosclerosis, aortic aneurysms and chronic obstructive pulmonary disease. Here we demonstrate a novel and major role for MMP-12 in the regulation of the innate antiviral response via effects on intracellular and circulating alpha interferon (IFN-alpha). Coxsackievirus type-B3 (CVB3) infected MMP-12 knockout mice (KO) exhibited high viral loads and mortality 3 days post infection, with low plasma IFN-alpha concentrations in comparison to wild-type (WT) counterparts. However IFN-alpha accumulation within KO cells in multiple organs suggested that its secretion is inhibited in the absence of MMP-12. Intravenous administration of IFN-alpha to MMP-12 KO mice reduced viral loads but resulted in a toxic unchecked immune response, suggesting that MMP-12 is required for inactivation of circulating IFN-alpha during antiviral immunity. Taken together, our results suggest that a checkpoint has evolved to prevent secretion and uncontrolled IFN-alpha activity in the absence of MMP-12 expression.

Abstract # 69

Sohrab Shah

S. P. Shah[1], M. Koebel[1], R. Morin[2], M. Sun[1], R. Giuliany[1], S.E. Kalloger[1], J. Senz[1], N. Boyd[1], K. Wiegand[1], E. Yorida[1], A. Zayed[1], I. Schrader[1], K. Tse[2], M. Hirst[2], S. Aparicio[1], C. B. Gilks[1], M. Marra[2], and D. G. Huntsman[1]

[1]British Columbia Cancer Agency, Vancouver BC Canada, [2]Genome Sciences Centre, British Columbia Cancer Research Centre, Vancouver BC Canada

Profiling ovarian carcinoma subtypes with next generation sequencing

Backround/Objectives

The ovarian carcinoma subtypes include type I carcinomas (low grade serous, mucinous, clear cell, and endometriod) and type II carcinomas (high grade serous (HGS) carcinomas). The latter represent 70% of all cases. These pathologically identifiable cancers have different epidemiologic and genetic risk factors, precursor lesions, biomarker profiles, and clinical behaviour. We have recently shown that the ovarian carcinoma subtypes are so distinct that grouping them together in studies aimed at identifying biomarkers that associate with stage or clinical behaviour will almost invariably give misleading results.

Methods

We interrogated the transcriptomes of 17 representative cases of three ovarian carcinoma subtypes (1 high grade serous, 5 clear cell, 4 granulosa cell, 4 endometrioid, 2 mucinous and 1 low grade serous cell line) with Illumina based paired end sequencing.

Results

At least 8 million paired end reads of 42 base pairs were obtained from each case. In total, we successfully aligned 22.4Gb of sequence to the reference genome.

Conclusion

Through computational analysis, our results indicate the presence of expected subtype-specific expression signatures, novel subtype specific mutations (single nucleotide variants (SNV) and insertions and deletions (indels)) and the presence of gene fusions in HGS carcinomas. Gene fusions were validated using RT-PCR and fluorescence in-situ hybridization (FISH). Genomic breakpoints were supported by genome-wide copy number analysis. SNVs and indels are currently being validated and followed up for clinico-pathologic significance.

Sohrab P. Shah[1], Rodrigo Goya[1], Mark G.F. Sun[1], Gavin Ha[1], Ryan Morin[2], Kim Wiegand[1], Kevin Murphy[3], Sam Aparicio[1], David Huntsman[1]

[1]British Columbia Cancer Agency, Vancouver BC Canada [2]Genome Sciences Centre, British Columbia Cancer Research Centre, Vancouver BC Canada [3]Dept of Computer Science, UBC, Vancouver BC Canada

Sohrab Shah

Evaluation of a bayesian mixture model for detection of single nucleotide variants in ovarian cancer transcriptomes by next generation sequencing

Backround/Objectives

The advent of next generation sequencing propelled the field of cancer genomics forward such that it is now cost-effective to interrogate entire transcriptomes of clinical tumor samples for the presence of somatic mutations. This approach generates a massive number of short sequence reads which once aligned to a reference human genome can reveal positions containing single nucleotide variants (SNVs). These are positions in a transcriptome or genome for which at least one allele differs from the reference human genome. The challenge in SNV detection is that the alleles are represented by a stochastic distribution of allelic counts in the aligned reads.

Methods

To model this distribution and infer the presence of SNVs, we developed a novel Bayesian mixture model called SNVmix. The model assumes the allelic counts at each position were generated from one of three genotypes, each of which 'emit' data with a specific Binomial distribution. We implemented an expectation maximization algorithm that simultaneously learns the parameters of the Binomial distributions, the prior distribution over genotypes, and the genotypes themselves for each position. We also implemented a version of the model that can account for uncertainty in the base call and the alignments of the reads. We quantitatively compared these models to each other and to a state of the art, and commonly used method for calling SNVs called MAQ.

Results

In this study we show the results of SNVmix applied to 16 ovarian cancer data sets derived from clinical samples. To evaluate the model, we generated Affymetrix SNP 6.0 high density genotyping arrays and genotyped the samples. Using positions from the array at which a genotype could be confidently called as ground truth, we computed receiver operator characteristic curves to evaluate the accuracy of our model. We also show a comparison to a state of the art method called MAQ on this same data, and how the characteristics of the input data such as sequence depth affect our results. Finally, we show clinically relevant SNVs in ovarian cancer in Beta-catenin, PTEN, PIK3CA and P53 discovered with SNVMix that have been experimentally validated.

Conclusion

We have demonstrated that the SNVmix model is more accurate than MAQ and represents a mathematically prinicpled, probabilistic model to detect SNVs from next generation sequencing data. This model is currently being applied in the analysis of 50+ ovarian transcriptomes and 70+ breast cancer genomes and transcriptomes.

Abstract # 71

Emma Strong (4th year student)

E. Strong, S. Martell, C. Harvard, Y. Qiao, E. Rajcan-Separovic

Dept of Pathology and Laboratory Medicine, UBC

Expression of brain function related genes in peripheral blood

Backround/Objectives

Intellectual Disability (ID) is a disease that affects between 1.5-2% of world population, yet advances in finding the cause and treatment for this disease is limited by the inaccessibility of relevant brain tissue. The objective of this study was to assess the possibility of using RNA from blood to monitor the expression of brain function related genes. Different extraction methods and different preparations of peripheral blood were used to obtain RNA and to assess the expression of a selection of brain genes in controls and in a child with ID.

Methods

In this study, peripheral blood from normal individuals was used to assess the possibility of detecting the expression of 7 brain related genes (FMO5, BCL9, ACP6, PRKAB2, CHD1L, GJA5 and GJA8) from a chromosomal region 1q21.1 which is known to show abnormalities (i.e gene copy number changes) associated with ID. Reverse transcription PCR (RT-PCR) was used to assess the expression. RNA was extracted from whole blood, stimulated short and transformed long term cultures of whole blood, expected to be enriched in T and B cell populations respectively. Additionally, RT-PCR was used to assess the expression of the selected genes in RNA extracted from blood of a child with ID, who had a subtle gain of chromosomal 1q21.1 (microduplication), and his unaffected mother. To assess the role of RNA preparation on RNA quality, several samples were subjected to post-RNA extraction DNase-1 treatment.

Results

Results indicated that whole blood was likely the best candidate for further expression studies of brain related genes as the expression of most genes was detected in a pattern that resembled their detection in control brain RNA. Short and long term culture did not allow for good detection of 1 of 7 genes (FMO5) and long term cultures showed suboptimal detection levels of ACP6. Little expression level difference between the proband with the 1q microduplication and his mother was detected for 3 of 4 genes within the 1q duplication, using RT-PCR. The only gene that showed a stronger expression in the long term blood culture of the proband compared to his mother was ACP6.

Conclusion

In conclusion, this study showed a) that blood can be used to study RNA expression, as 5 of 7 brain function related genes selected had detectable expression in blood, b) RNA extracted from whole blood provides consistent and strong gene expression levels for the studied genes, c) the RNA preparation method affects the detection of expression of the studied genes and DNase treatment is preferred during rather than after RNA extraction and d) the increase in gene copy number due to a gene duplication tends not to show a detectable increase in expression and was detectable for 1 of 4 tested genes when simple RT-PCR was used. Other quantification methods should be used, for example quantitative real time PCR to determine the level of gene expression more accurately and is the aim of our future investigations.

Chris M.J. Conklin[1], Sohrab P. Shah[2], Mark Sun[2], Nataliya Melnyk[2], David Huntsman[2]

[1]Dept of Pathology, University of British Columbia, Vancouver, BC, [2]Centre for Translational and Applied Genomics of British Columbia Cancer Agency and the Provincial Health Services Authority Laboratories, Vancouver, BC

Chris Conklin

Abstract # 72

Ovarian small cell carcinoma hypercalcemic type: exploring the genetic basis for this aggressive neoplasm

Backround/Objectives

Ovarian small cell carcinoma of the hypercalcemic type (OSCCHT) is a rare, highly aggressive tumor of uncertain histological origin. The tumor occurs almost exclusively in young females and has a poor prognosis. Two-thirds of patients have elevated serum calcium levels. Thus, PTH, calcitonin, and other genes involved in calcium signaling may underlie the pathogenesis of OSCCHT. Alternatively, novel mutations or chromosomal aberrations may drive its growth. Improved understanding of the genetic basis for OSCCHT could lead to new targeted therapies.

Methods

DNA was extracted from a xenograft model of human OSCCHT (BIN-67) and fluorescent in situ hybridization (FISH) was used to identify any structural abnormalities present in the chromosomal sequences. BIN-67 cell line was interrogated using whole-transcriptome paired-end RNA sequencing. Single nucleotide variants (SNV) and small insertions and deletions were identified and cross-referenced against human genome databases to eliminate previously described germline variants. The variants were confirmed by direct sequencing of cDNA and genomic DNA (gDNA) using index cases of OSCCHT derived from the OvCaRe frozen tumor bank at the Vancouver General Hospital.

Results

Chromosomal analysis did not reveal any significant deletions, duplications, amplifications, or structural abnormalities. Transcriptome analysis is currently ongoing. However, preliminary results reveal point mutations in genes associated with Wnt, p53 and MAPK pathways. None of the most common mutations seen in other ovarian tumors (KRAS, BRAF, CTNNB1, CDKN2A, PI3K3CA, BRCA1, BRCA2) are present on initial study.

Conclusion

Based on chromosomal analysis, OSCCHT appears to be a point mutation driven tumor. Preliminary results suggest that OSCCHT may be genetically unique compared to other ovarian tumors. Results are ongoing.

Abstract # 73

Ying Qaio

Ying Qiao[1], Tyson Christine[2], Laura Arbour[3], Francois Bernier[4], Chansonette Harvard[1], X Liu[5], Celina Fawcett[2], Martin Somerville[6], Jeanette Holden[5], Suzanne Lewis[7], Evica Rajcan-Separovic[1]

[1]Dept of Pathology, UBC, [2]Cytogenetics Lab, Royal Columbian Hospital, [3]Medical Genetics, Victoria, [4]Medical Genetics, Calgary; [5]Queens University, Kingston, [6]Molecular Diagnostic Laboratory, Edmonton, [7]Medical Genetics, Vancouver

Interpretational challenges OF Xp22.31 duplication

Chromosomal deletions involving the Xp22.31 region are known to be associated with intellectual disability (ID); however, specific genes which may play a role in ID are not yet known. The most frequently suspected candidates (members of the VCX gene family, and neuroligin 4) were recently shown to be deleted in both affected and unaffected males. The phenotypic role of Xp22.31 micro-duplication is even less certain, mainly due to the small number of reported cases. Here, we describe two males with overlapping micro-duplications within Xp22.31, detected using a 1 Mb resolution BAC array (Spectral Genomics), and further confirmed and refined using 0.01 Mb higher resolution oligo arrays (Agilent). In both patients, the duplication was maternal in origin; in Family 1 it was transmitted by a phenotypically normal grandfather, while in Family 2 it was transmitted from the grandmother. The mothers of both probands showed skewed X inactivation (92% and 96%). Both of our two subjects and two previously described subjects with ID and Xp22.31 microduplication (Tyson et al., 2005 and Wagenstaller et al., 2007) have severe language delay/apraxia in common. The combination of the probands' abnormal phenotypes, in addition to the maternal skewed X inactivation, suggests a pathogenic role of the Xp22.31 micro-duplication. However, its presence in an unaffected male family member indicates that it is a benign change. The comparison of affected genomic regions and clinical features seen in the current and previously reported ID subjects with Xp22.31 duplication will be presented, and the possible causes of different phenotypic expression of the Xp22.31 duplication (i.e. imprinting, unrelated X-linked abnormalities, variable penetrance) will be discussed.

Ronald F Donnelly[1], Elaine Willman[2], Gary Andolfatto[3]

[1]Dept of Pharmacy, The Ottawa Hospital, Ottawa, Ontario, [2]Deptt of Pathology, University of British Columbia, Vancouver, BC, [3]Dept of Emergency, Lion's Gate Hospital, North Vancouver, BC

Elaine Willman

Stability of ketamine-propofol mixtures for procedural sedation and analgesia in the emergency department

Backround/Objectives

The mixture of ketamine and propofol administered together is reportedly an effective agent for procedural sedation and analgesia in the emergency department. However, the physical compatibility and chemical stability of extemporaneous solutions prepared from generic formulations of the drugs are not known. The objective is to investigate the physical compatibility and chemical stability of 50:50 and 30:70 mixtures of generic ketamine and propofol packaged in polypropylene syringes and stored at room temperature with exposure to light.

Methods

Mixtures of ketamine (10 mg/mL) and propofol emulsion (10 mg/mL) were prepared at 50:50 and 30:70 ratios, packaged in capped polypropylene syringes (3 syringes for each mixture), and stored at room temperature with exposure to light. One sample from each syringe was analyzed in duplicate at time 0 and after 1 and 3 hours. Physical changes such as pH, separation or cracking of the emulsion, change in colour, and formation of gas were monitored. The chemical stability of each drug was assessed by high-performance liquid chromatography.

Results

Both mixtures of ketamine and propofol were physically compatible during storage for up to 3 hours. There were no signs of change in any of the physical parameters during the 3-hour study. Each drug retained at least 97% of its original concentration.

Conclusion

Mixtures of ketamine and propofol at 50:50 and 30:70 ratios were physically compatible and chemically stable for up to 3 hours when stored in capped polypropylene syringes at room temperature with exposure to light.

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G227 - 2211 Wesbrook Mall
Vancouver, BC V6T 2B5
tel 604-822-7102; fax 604-822-9703
www.pathology.ubc.ca