Pathology Day

May 25, 2012

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ABSTRACT BOOK / POSTERS & ORAL PRESENTATIONS

a place of mind

THE UNIVERSITY OF BRITISH COLUMBIA

pathology laboratory medicine

ACKNOWLEDGEMENT

Jacqueline Quandt, BSc, PhD (UBC)

Michael Nimmo, BA, BSc Honours, LLB, MD (UBC), LMCC, FRCPC

Pathology Day is a team effort and we would like to extend our thanks to all those who contributed to the 2012 edition. These include Michael Allard, Maureen Barfoot and Adeline Chan, for their help in organizing this event. A special thanks to Debbie Bertanjoli for the website tools and the preparation of the abstract book. We also wish to express our gratitude to the many people who contributed their time and expertise reviewing the abstracts, moderating the oral sessions, and judging the oral, science bytes, and poster presentations. Finally, sincere thanks to all the volunteers who assisted with technical and administrative support

We hope you enjoy Pathology Day 2012.

Jacqueline Quandt and Michael Nimmo
Co-Chairs, Pathology Day 2012

SPECIAL THANKS TO THE MANY FACULTY WHO HAVE CONTRIBUTED AS JUDGES OR CHAIRS FOR PATHOLOGY DAY:

Blake Gilks
Colby Zaph
Diana Ionescu
Katerina Dorovini-Zis
Michael Angelsio
Wes Schreiber
Olena Maydan
Magda Martinka
Maria Monsalve
Amanda Bradley
Helene Cote
Maria Issa
Haydn Pritchard
Cheng-Han Lee
Cedric Carter
John English
Cheryl Wellington
Jacqui Brinkman
Susan Porter
Michael Allard
Pat Doyle
Marianne Sadar
Mike Nimmo

WE WOULD ALSO LIKE TO RECOGNIZE THE TECHNICAL AND ADMINISTRATIVE SUPPORT OF SEVERAL DEPARTMENT MEMBERS INCLUDING:

Jenny Tai
Jennifer Xenakis
Tony Lin
Andrew Leung
Jackie Leung

A MESSAGE FROM THE HEAD

WELCOME

Michael F. Allard, BSc, MD, FRCP(C)
Professor and Cardiovascular Pathologist, UBC
James Hogg Research Centre
Heart + Lung Institute
St. Paul's Hospital

Pathology Day is a critically significant event in the departmental calendar as it serves as a time to showcase scholarly activities, including basic investigative, translational and clinical-applied research, performed by our trainees and, by extension, our faculty. This gathering also provides a perfect venue to recognize and acknowledge the many outstanding contributions by members of the department over the past year.

Pathology Day also serves another critically important function. Specifically, it is an opportunity to get together to socialize and learn about one another as well as gain an appreciation and understanding for the breadth of scholarly activities that take place in our geographically dispersed department. We are very fortunate to have two outstanding scientists participate in the program this year, highlighting research excellence and continuing in the tradition of having world leaders in biomedical research speak at Pathology Day. Dr. Katerina Dorovini-Zis (Department of Pathology and Laboratory Medicine, University of British Columbia) will give the James Hogg Lecture, while Dr. Marco Marra (Department of Medical Genetics, University of British Columbia) is our Keynote Speaker. I extend my thanks and gratitude to Drs. Jacquie Quandt and Mike Nimmo, Co-Chairs of Pathology day, as well as all the other individuals for their efforts in organizing this year's event.

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KEYNOTE LECTURE

MARCO A. MARRA, PhD, FRS(C), FCAHS, OBC

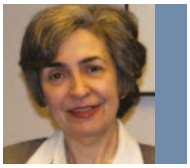
DIRECTOR & DISTINGUISHED SCIENTIST, GENOME SCIENCES CENTRE, BC CANCER AGENCY
PROFESSOR, DEPARTMENT OF MEDICAL GENETICS, UNIVERSITY OF BRITISH COLUMBIA ADJUNCT PROFESSOR, DEPARTMENT OF MOLECULAR BIOLOGY AND BIOCHEMISTRY, SFU UBC CANADA RESEARCH CHAIR IN GENOME SCIENCE

TITLE: "DECODING CANCERS"

Dr. Marra completed his PhD in Genetics at Simon Fraser University in 1994 and subsequently worked as a postdoctoral fellow and research faculty instructor at Washington University in St. Louis. He has been involved in the development and application of efficient, high-throughput genomics approaches, with special emphasis on large-scale genome mapping and DNA sequencing.

Current activities include the development and application of "next generation" sequencing approaches to characterize genomes, with the aim of comprehensive identification of the genetic changes that drive cancer progression. Dr. Marra is the UBC Canada Research Chair in Genome Science, and a member of the Order of British Columbia. He is a recipient of a 2010 Genome BC Award for Scientific Excellence. He was elected to the Canadian Academy of Health Sciences in 2009; received the Frontiers in Research Award from the BC Innovation Council in 2008; and was appointed a Fellow of the Royal Society of Canada in 2007. He was a recipient of a Distinguished Achievement Award from UBC, a MSFHR Career Investigator Senior Scholar Award, and Simon Fraser University President's 40th Anniversary Award. In 2004, he received a Terry Fox Young Investigator Award and BC Biotech's Innovation and Achievement Award (together with the entire GSC staff) for sequencing the SARS coronavirus genome. Dr. Marra's contributions to genome science led to an honorary Doctor of Science degree from Simon Fraser University in 2004, and an honorary Doctor of Laws degree from the University of Calgary in 2005.

Dr. Marra has published 12 book chapters and 233 peer-reviewed papers.



KATERINA DOROVINI-ZIS, MD, FRCPC

PROFESSOR OF PATHOLOGY AND LABORATORY MEDICINE AT THE UNIVERSITY OF BRITISH COLUMBIA & HEALTH SCIENCES CENTER

TITLE: "CEREBRAL MALARIA IN CHILDREN: A NEUROVASCULAR DISORDER"

Dr. Dorovini-Zis earned her M.D. degree form the National University of Athens, Greece. She completed a residency in anatomical pathology and neuropathology at Vancouver General Hospital. After a research fellowship at the National Institutes of Neurological Disorders and Stroke, NIH, supported by a Fogarty Fellowship (1977-1980), she joined the faculty of the University of Michigan in 1980. She joined the faculty of the University of British Columbia in 1985.

Dr. Dorovini-Zis has held memberships with many organizations. She was the recipient of the Women in Neuroimmunology Award for her contributions to the field of Neuroimmunology, at the Vth International Congress of Neuroimmunology in 1998. She has authored and co-authored over 70 publications and several book chapters. She has served on grant review committees for the Multiple Sclerosis Society of Canada, the Canadian Heart and Stroke Foundation and the Alberta Health Foundation for Medical Research and as editorial board member in several journals. She has been actively involved in undergraduate, graduate and post-graduate teaching. She worked as Consultant Neuropathologist at Vancouver General Hospital and served as Head of the Division of Neuropathology from 1994 to 2007. She is the director of the Neuropathology Research Laboratory.

Dr. Dorovini-Zis' primary research interest is to understand the mechanisms of leukocyte recruitment across the blood-brain barrier in central nervous system inflammation, focusing on the role of the cerebral endothelium as an active participant in this process. Upon establishing her independent Neuropathology Research Laboratory in 1985, she created the first in vitro model of the human blood-brain barrier that mimics the blood-brain barrier in vivo. This model has been used in her laboratory to investigate the mechanisms that support adhesion and migration of different leukocyte subtypes (lymphocytes, monocytes, neutrophils, dendritic cells) across the blood-brain barrier and determine the effects of inflammatory mediators on barrier permeability and on how endothelial-derived adhesion molecules, class II MHC, costimulatory molecules and chemokines mediate leukocyte-endothelial cell interactions at the blood-brain barrier. Her interests extend to the study of blood-brain barrier dysfunction in infectious and inflammatory CNS diseases with particular emphasis on the mechanisms responsible for blood-brain barrier dysfunction and brain damage in pediatric cerebral malaria.

08:00AM	Breakfast (Atrium)
08:25AM	Opening remarks: Mike Allard, Professor and Head, Dept of Pathology & Laboratory Medicine
08:30AM	JAMES HOGG LECTURE, LECTURE THEATRE
	DR. KATERINA DOROVINI-ZIS "Cerebral Malaria in Children: A Neurovascular Disorder"
09:30AM	**SCIENCE BYTES** Opening remarks: Jacqueline Quandt
10:45AM	15 min break (Atrium)

RESIDENT ORAL SESSION (CHAIR: MICHAEL NIMMO) MULTIPURPOSE ROOM

ABSTRACT #		
1.	11:00AM	HIJAZI, NOUF ARIfrequent in grade 1 endometrioid carcinoma of the endometrium than in complex atypical hyperplasia.
2.	11:15AM	LEO, JOYCE Lineage-specific markers are rarely expressed in basal-like breast cancer.
3.	11:30AM	HUANG, JINGYANG EGFR protein expression and genetic amplification in high-grade pleomorphic uterine sarcomas.
4.	11:45AM	GURUNG, ANANTA Diagnostic pitfalls in assessment of tumour type, regression and stage in patients with locally advanced low rectal cancer following pre-operative chemo- radiotherapy.
	12:00PM	Lunch (Atrium)
	12:30PM	**POSTER PRESENTATIONS, ATRIUM**
5.	2:00PM	MORIN, JASON Angiotropism in primary melanoma with brain metastases: a study of 20 cases.
6.	2:15PM	MOHAMMAD, TAREQ A real-world test of cea, cd15, calretinin, and ck5/6 for distinguishing between mesotheliomas and pulmonary adenocarcinomas.
7.	2:30PM	CONKLIN, CHRIS Immunohistochemistry may be a reliable screening tool for identification of ALK rearrangement in non-small cell lung cancer.
8.	2:45PM	WONG, PATRICK Performance of various Autoantibody Immunoassays in screening for and diagnosis of Systemic Autoimmune Rheumatic Diseases.
	3:00PM	15 min break (Atrium)
9.	3:15PM	WILMER, AMANDA Methicillin-resistant staphylococcus aureus strain usa300 is prevalent among hospital-onset cases in an urban canadian setting.
10.	3:30PM	FRYKMAN, HANS A prospective longitudinal, study of the humoral immune responses in women infected with oncogenic HPV strain(s) to the cervix using protein microarrays.
11.	3:45PM	DAVANI, EHSAN The role of ICAM-1 activation in myocardial dysfunction during sepsis.
12.	4:00PM	SEKIROV, INNA Salmonella-mediated neutrophil recruitment in murine colitis alters the host intestinal microbiota via neutrophil elastase activity.

GRADUATE STUDENT AND PDF ORAL SESSION (CHAIR: MARIANNE SADAR) LECTURE THEATRE

ABSTRACT #		
13.	11:00AM	HIEBERT, PAUL Impaired wound healing in apolipoprotein e deficient mice.
14.	11:15AM	HENDEL, ALON Granzyme B cleavage of fibronectin leads to reduced endothelial cell adhesion migration and capillary tube formation.
15.	11:30AM	NEIL, SARAH A radical new therapy for multiple sclerosis? Immunomodulatory and neuroprotective properties of the antioxidant TEMPOL.
16.	11:45AM	MARWAHA, ASHISH Tregs from T1D subjects with a susceptible IL-2R gene SNP, respond aberrantly to IL-2 stimulation.
	12:00PM	Lunch (Atrium)
	12:30PM	**POSTER PRESENTATIONS, ATRIUM**
17.	2:00PM	CHUNG, BRIAN K Natural killer T cells control B cells infected and transformed by epstein-barr virus.
18.	2:15PM	YE, XIN microRNA(miR)-126 promotes coxsackievirus B3 induced cell death by targeting Wnt signaling pathway.
19.	2:30PM	SOHI, GURMEET KAUR Measuring mitochondrial DNA oxidative damage by HIV nucleoside reverse transcriptase inhibitors in cultured cell models.
20.	2:45PM	ZANET, DEANNA Factors associated with shorter leukocyte telomere length in HIV+ and HIV- adults.
	3:00PM	15 min break (Atrium)
21.	3:15PM	TAVAKOLI, IRAN Inhibition of castration resistant prostate cancer by sintokamide A - a novel antagonist of the amino-terminus of the androgen receptor.
22.	3:30PM	EL NAGGAR, AMAL Y box protein 1: a novel direct translational activator of hypoxia inducible factor 1α steering sarcoma cell invasion & metastatic dissemination.
23.	3:45PM	YANG, KEVIN Characterization of androgen receptor-targeting small molecule terpenes for prostate cancer.
24.	4:00PM	HARBOURNE, BRYANT THOMAS The role of hypoxia induced secreted proteins in breast cancer metastasis..

4:30PM KEYNOTE LECTURE, LECTURE THEATRE

DR. MARCO MARRA "Decoding Cancers"

PATHOLOGY DAY AWARDS & GALA DINNER

6:00PM **SHAUGHNESSY RESTAURANT AT VANDUSEN GARDENS, 5251 OAK STREET**

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ABSTRACT #

ARID1A LOSS OF EXPRESSION IS MORE FREQUENT IN GRADE 1 ENDOMETRIOID CARCINOMA OF THE ENDOMETRIUM THAN IN COMPLEX ATYPICAL HYPERPLASIA

N Hijazi[1], D Huntsman[2], B Gilks[3]

[1]Department of Pathology and Laboratory Medicine, UBC; [2,3] The Center for Translational and Applied Genomics (CTAG) at the British Columbia (BC) Cancer Agency

Background /objectives

ARID1a is a tumor suppressor gene, lost in many gynecological cancers, that is implicated in various chromatin remodeling processes, via its encoded protein BAF250.

Methods

We studied a total of 40 cases, which comprised 17 cases of complex atypical hyperplasia (CAH), 11 cases of grade 1 endometrioid adenocarcinoma of the endometrium (Endo Ca) and 12 cases of CAH mixed with Endo Ca (Mixed). Corresponding paraffin embedded formalin fixed blocks were retrieved and ARID1a staining (BAF250a mouse clone 3H2, Abgent, San Diego, CA, USA) was performed on whole tissue sections and assessed based on the presence or absence of nuclear staining.

Results

In the CAH group, ARID1a staining was retained in 13 cases (76%) with focal (partial) loss in 3 cases (18%) and complete loss in 1 case (6%). Of the 11 Endo Ca cases, 6 retained ARID1a expression (55%), four showed partial loss (36%) and one exhibited complete loss of expression (9%). In the Mixed group, 3 CAH (25%) and 6 Endo Ca (50%) demonstrated loss of expression, manifesting as either partial or complete loss. Partial loss of expression was manifested as areas within the CAH or Endo Ca showing loss of expression in all epithelial cells within the contiguous area of loss (so-called clonal pattern of loss).

Conclusions

Loss of ARID1a expression occurs in endometrial preneoplastic lesions, i.e. complex atypical hyperplasia, albeit at a low frequency (24-25%) with more frequent loss seen as carcinoma develops (45-50%).ARID1a can be lost at either the premalignant phase (CAH) or after carcinoma has already developed (i.e. during tumor progression).

LINEAGE-SPECIFIC MARKERS ARE RARELY EXPRESSED IN BASAL-LIKE BREAST CANCER

Joyce M Leo[1], Jennifer Choo[1], Allen M Gown[1,2], Torsten O Nielsen[1], C Blake Gilks[1]

[1]University of British Columbia, Department of Pathology, Vancouver, Canada; [2]Phenopath Laboratories, Seattle, WA, United States

Background /objectives

Basal-like breast cancer is defined by expression profiling, and is an aggressive subtype accounting for approximately 15% of breast cancer cases and a disproportionately high percentage of breast cancer metastases (particularly to lung and brain) and deaths. Basal-like breast cancers rarely express the common breast cancer biomarkers estrogen receptor, progesterone receptor and HER-2. In this study we sought to characterize their expression of other commonly-used diagnostic immunomarkers of carcinoma cell lineage, based on the hypothesis that they will be more likely to show aberrant expression of lineage-specific markers (i.e. decreased expression of breast cancer markers and increased expression of markers of non-breast lineage).

Methods

Basal-like breast cancers, defined by PAM50 expression profiling, were placed in a tissue microarray and immunostained for mammaglobin, GCDFP-15, WT1, TTF1, CDX2, napsin A, PAX2, PAX5 and PAX8.

Results

TTF1, WT1, CDX2 and napsin A were negative in all cases. Among commonly used markers of the breast lineage, mammaglobin was positive in only 2/46 and GCDFP-15 was positive in just 1/46 of the basal-like breast cancers tested. PAX2 and PAX5 were positive in 1/46, while PAX8 was positive in 2/46 cases of basal-like breast carcinomas.

Conclusions

Basal-like breast cancers show a very low frequency of expression of the breast cancer markers mammaglobin and GCDFP-15, limiting their usefulness as markers of site of origin when one is examining metastatic carcinoma of unknown primary and metastatic basal-like breast cancer is a consideration. Basal-like carcinomas only rarely express antigens used as markers of non-breast origin, such as WT1, TTF1, CDX2, napsin A, PAX2, PAX5 and PAX8. Better positive markers are needed for identification of basal-like breast cancer in the metastatic setting.

EGFR PROTEIN EXPRESSION AND GENETIC AMPLIFICATION IN HIGH-GRADE PLEOMORPHIC UTERINE SARCOMAS

Jingyang Huang[1], Cheng-Han Lee[2], Adam Smith[3]

[1],[2]Department of Pathology and Laboratory Medicine, Vancouver General Hospital; [3] BC Cancer Agency, Vancouver Centre

Background /objectives

Epidermal growth factor receptor (EGFR) is known to be frequently expressed in undifferentiated endometrial sarcomas (UES) and the sarcomatous component of malignant mixed mullerian tumors (MMMT or carcinosarcoma), but little is known regarding its genetic basis. Only a single case of UES with EGFR amplification which showed a significant but temporary response to imatinib has been previously reported (Ann Diagn Pathol. 2007;11(1):49-54). The aim of the current study is to evaluate EGFR copy number and amplification status in a series of non-smooth muscle high-grade pleomorphic uterine sarcomas.

Methods

We identified a series of 14 high-grade non-smooth muscle primary uterine sarcomas (hysterectomy specimens) through retrospective review of pathology archive at Vancouver General Hospital since 2000. All cases demonstrated high-grade nuclear features with diffuse and marked nuclear pleomorphism and none showed features of endometrial stromal sarcoma. These included 8 UES, 4 uterine sarcomas with rhabdomyosarcomatous differentiation (US-RMS), 1 uterine sarcoma with osteosarcomatous differentiation (US-OSA) and 1 adenosarcoma that is nearly completely overgrown by high-grade undifferentiated sarcomas (US-AD). EGFR (Dako) immunostaining was performed on representative whole sections and scored using Her-2/neu scoring criteria for breast cancer. Fluorescence in situ hybridization (FISH) for EGFR and CEP7 (LSI EGFR/CEP7, Abbott Molecular, 40 tumor nuclei evaluated) was performed in cases that showed 3+ EGFR protein expression.

Results

Seven of the 14 cases showed 3+ EGFR protein expression (4 UES, 2 US-AD, 1 US-RMS and 1 US-OSA). Interpretable FISH was obtained in five cases with 3+ EGFR staining with the remaining two cases to be repeated (data pending). Among these 5 cases, one case (US-OSA) showed EGFR amplification (EGFR/CEP7 ratio 2.07) while the 3 other cases (1 UES, 1 US-RMS and 1 US-AD) showed increased EGFR copy numbers in >50% of the nuclei evaluated with similar corresponding increase in CEP7 signals.

Conclusions

A significant subset of non-smooth muscle high-grade uterine sarcomas strongly expresses EGFR and the data here suggest that EGFR amplification or increased copy number appear to be the underlying genetic mechanism for the observed protein expression. These findings, together with the prior reported case of EGFR-amplified UES provide a compelling rationale for therapeutic targeting of EGFR in these high-grade uterine sarcomas.

DIAGNOSTIC PITFALLS IN ASSESSMENT OF TUMOUR TYPE, REGRESSION AND STAGE IN PATIENTS WITH LOCALLY ADVANCED LOW RECTAL CANCER FOLLOWING PRE-OPERATIVE CHEMO-RADIOTHERAPY

Ananta Gurung[1], Stephen Yip[2], Hagen Kennecke[3], Chen Zhou[2]

[1]Department of Pathology and Laboratory Medicine, Vancouver General Hospital; [2]Department of Pathology and; [3]Department of Medical Oncology, BC Cancer Agency

Background /objectives

Locally advanced colorectal adenocarcinomas are commonly treated with pre-operative chemoradiotherapy (CRT). Neo-adjuvant CRT downstages patients, induces tumour regression and induces pathological complete response (pCR) in approximately 20%, which is associated with improved clinical outcomes. With a myriad of histological patterns of tumour response (including necrosis, fibrosis, acellular mucin pools), accurate staging (pTNM and ypTNM) can be challenging. The aim of this study was to evaluate the pathological response and identify diagnostic pitfalls in patients with locally advanced low rectal cancer following pre-operative CRT.

Methods

42 patients were enrolled in a phase II trail of pre-operative bevacizumab, capecitabine, oxaliplatin and radiation. Slides and tumour blocks from 38 of these patients who went onto definitive surgery were retrieved from referring hospitals and central review of slides was performed. Tumor regression was graded using the Dworak system (Grade 0 = no regression, 1 = <25% of tumour mass, 2 = ≥25-50%, 3 = ≥50%, 4 -complete tumour regression), histological patterns of tumour response and reasons for changes to pathological stage on central pathology review were analyzed.

Results

pCR was seen in 18% (7/38) of patients. Central review resulted in change in pathological stage rendered previously in 16% (6/38) of cases, with two patients being upstaged, and 4 downstaged. All cases following CRT showed some degree of necrosis and fibrosis. Acellular mucin pools (6%, 3/38) and mucin pools surrounded by neoplastic glands (26%, 10/38) were identified as common reasons for changes to pathological stage. Furthermore, these two histological findings resulted in reclassification of tumour subtype, as mucin pools surrounded by adenomatous glands were misinterpreted as mucinous adenocarcinoma (11%, 4/38).

Conclusions

Histological responses to pre-operative CRT present challenges to diagnostic surgical pathologists. To render accurate diagnoses, stage and valuable prognostic information, resected specimens showing mucin pools should be interpreted with caution.

ANGIOTROPISM IN PRIMARY MELANOMA WITH BRAIN METASTASES: A STUDY OF 20 CASES

Jason Morin[1], Tawny Hung[1], Ian MacKenzie[1] and Raymond Barnhill[2]

[1]Departments of Pathology & Laboratory Medicine, UBC; [2]UCLA

Background /objectives

Direct vascular invasion and hematogenous spread of tumour cells is a frequent mechanism involved in tumour metastases. However, increasing studies show that an alternate mechanism may be important in the spread of melanoma. Based on observational studies it has been suggested that melanoma cells can migrate along the external surface of vessels, a process that has been termed extravascular migratory metastasis (EVMM). Angiotropism, defined as the presence of malignant cells abutting the outsides of vessels at the advancing edge of the tumor, is a histological surrogate for EVMM.

Methods

In this study, we retrospectively investigated 20 primary melanoma cases and their respective metastatic brain lesions. The following parameters were analyzed in each primary melanoma case: presence of angiotropism, Clark level, Breslow depth, mitotic activity, sentinel lymph node status, time interval between primary lesion and metastasis, and the age and sex of the patient. The metastatic brain lesions were also examined for the presence of angiotropism.

Results

Of the 20 cases, 11 showed angiotropism in the primary melanoma. The average age of the angiotropic group was 51-years-old; the average age in the non-angiotropic group was 57-years-old. The angiotropic group had a significantly deeper Breslow thickness (4.44cm vs 1.41cm, p= 0.00016) and was more mitotically active (14.2 mitosis/mm2 vs 4.67 mitosis/mm2, p= 0.020). Importantly, the angiotropic group had an average time lapse of 31 months from the primary lesion to the brain metastasis, while the non-angiotropic group had a 57-month time interval (p=0.021). The presence of angiotropism in the brain metastasis, however, had no significant correlation with the presence of angiotropism in the primary lesion.

Conclusions

A total of ten of the cases had sentinel lymph node (SLN) biopsies performed; 6/10 cases were in the angiotropic group and 4/10 were in the non-angiotropic group. Three of the 6 cases in the angiotropic group were positive, and had an average time lapse of 32 months from the primary lesion to the brain metastasis. All four SLN biospies were positive in the non-angiotropic group, and these patients had an average 55-month time interval to brain metastasis. We herein present the first study of angiotropism in primary melanoma lesions and their relationship with melanoma brain metastasis.

A REAL-WORLD TEST OF CEA, CD15, CALRETININ, AND CK5/6 FOR DISTINGUISHING BETWEEN MESOTHELIOMAS AND PULMONARY ADENOCARCINOMAS

Tareq Mohammad[1], John Garratt[1], Emina Torlakovic[2], Blake Gilks[1], Andrew Churg[1]

[1]Departments of Pathology, Vancouver General Hospital and University of British Columbia; [2]University Health Network and University of Toronto

Background /objectives

Most reports on antibodies claimed to separate mesothelioma from pulmonary adenocarcinoma originate in academic centers or specialized immunohistochemistry laboratories, but little is known about how such stains perform in general practice laboratories.

Methods

The Canadian Immunochemistry Quality Control (CIQC) Program circulates tissue array slides to laboratories across Canada; these are stained, and then interpreted by the local laboratory and by a set of experienced reviewers. For CIQC run 16, tissue array slides from 16 pulmonary adenocarcinomas and 6 mesotheliomas were stained by 36 different laboratories for CEA, CD15, CK5/6 and calretinin.

Results

736 results ("cases") were interpretable. If 3 of 4 staining results concordant with the diagnosis was accepted as definitive, 166/192 (86.4%) mesotheliomas and 461/544 (84.7%) adenocarcinomas were correctly diagnosed. However, if 4 of 4 concordant markers were required, then 93/192 (48.4%) mesotheliomas and 265/544 (48.7%) adenocarcinomas were correctly diagnosed. Only 3/192 (1.6%) mesotheliomas were incorrectly classified as carcinomas and 8/544 (1.5%) of adenocarcinomas incorrectly classified as mesotheliomas based on the immunoprofile (i.e. 3 or 4 of 4 marker results were discordant with the diagnosis).

Conclusions

We conclude that, in a study based on results from nonspecialized laboratories, the combination of CEA, CD15, calretinin, and CK5/6, used as a panel, has a very low false positive rate when separating pulmonary adenocarcinomas from mesotheliomas; however, single incorrect results are common, so that the panel is only useful diagnostically if 3 of 4 "correct" results are deemed acceptable for diagnosis.

IMMUNOHISTOCHEMISTRY MAY BE A RELIABLE SCREENING TOOL FOR IDENTIFICATION OF ALK REARRANGEMENT IN NON-SMALL CELL LUNG CANCER

Chris MJ Conklin[1], Kenneth J Craddock[2], Cherry Have[2], Ming Tsao[2], Christian Couture[3], Diana N Ionescu[4]

[1]UBC, Anatomical Pathology; [2]University Health Network, Toronto General Hospital, University Health Network, Toronto General Hospital, Department of Pathology; [3]DIUCPQ (Hospital Laval); [4]BC Cancer Agency

Background /objectives

Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor that may undergo rearrangement to form a fusion gene (EML4-ALK) in a subset of non-small cell lung cancer (NSCLC) patients. The discovery of this fusion gene was a breakthrough in targeted therapy for lung cancer, with significant clinical implications. ALK rearrangement (ALKR) is however only seen in a small percentage of NSCLC making identification of these patients challenging and costly.

Methods

Using immunohistochemistry (IHC) with five antibody systems to ALKR (5A4 by Novocastra and Nichirei's N-Histofine ALK detection kit, and D5F3 by Cell Signalling Technology, and DAKO clone ALK1 by FLEX and ADVANCE), and fluorescence in situ hybridization (FISH), we screened 385 cases of stage I NSCLC in a tissue microarray format. IHC was scored as 0 (no staining), 1+ (faint cytoplasmic staining), 2+ (moderate, smooth cytoplasmic staining) and 3+ (intense, granular cytoplasmic staining) in >10% of tumor cells. IHC suspicious cases were 1+ and 2+. IHC positive cases were 3+ only. Suspicious and positive cases were confirmed by IHC and FISH on whole section (WS).

Results

Results by FISH were available on 273 cases and by IHC on 385 cases. The incidence of ALK rearrangement in this cohort was 1.1% as determined by FISH. After TMA screening, 11 cases were either suspicious by IHC (5) or FISH (6), and one case was suspicious by both methods. Using FISH-positivity on WS as the gold standard, D5F3 and 5A4 (by both Novocastra and Nichirei), each showed 100% sensitivity compared to DAKO clone ALK1 (by both ADVANCE and FLEX), which showed 66% sensitivity. Of the most sensitive antibody systems, D5F3 and 5A4 by Novocastra both showed 87.5% specificity, whereas 5A4 by Nichirei showed 62.5% specificity.

Conclusions

IHC presents a cost-effective and efficient means of identifying ALK-positive NSCLC patients but is highly dependent on the type of antibodies available. Certain ALK antibodies may not identify all cases with gene rearrangement by FISH. Among the commercially available antibodies, 5A4 by Novocastra and D5F3 provide the greatest combination of sensitivity (100%) and specificity (87.5%), and therefore are the most recommended antibodies to screen for ALKR in NSCLC.

ORAL PRESENTATION

PERFORMANCE OF VARIOUS AUTOANTIBODY IMMUNOASSAYS IN SCREENING FOR AND DIAGNOSIS OF SYSTEMIC AUTOIMMUNE RHEUMATIC DISEASES

Patrick Wong[1], David Pi[1,3], Mike Nimmo[1,3], Carmen Phoon[3], Jason Pal[3], Deidre O'Connor[2], Steven PJ[1] Rick White[4], Monika Hudoba de Badyn[1,3], Kam Shojania[2]

[1]Department of Pathology and Laboratory Medicine; [2]Department of Medicine, Faculty of Medicine, UBC; [3]Department of Pathology, Vancouver General Hospital; [4]Department of Statistics, UBC

Background /objectives

Anti-nuclear antibody by immunofluorescence (ANA-IF) is currently the gold standard screening procedure for Systemic Autoimmune Rheumatic Diseases (SARDs), but is both time consuming and subjective. Clinical laboratories routinely perform Extractable Nuclear Antigen-6 (ENA-6) and/or dsDNA on patients with high ANA titers to help subtype SARDs. In recent years, automation and technology have promoted the use of a one step testing strategy (ENA-6 or multiplex bead-based immunoassay (MPBI)) as an alternative to ANA-IF screening.

Objective/Methods

Part I - To evaluate the performance of several diagnostic algorithms in screening for SARDs (one step testing with ANA-IF, ENA-6, MPBI; two step reflex testing at various ANA titers). Part II - To evaluate the performance of ENA-6 and MPBI in subtyping SARDs.

Autoantibody assay results of 329 in-patients with identifiable clinical information were statistically evaluated.

Results

Part I - One step testing showed comparable sensitivities and positive predictive values among all 3 methods, but MPBI had the highest specificity followed by ENA-6 and then ANA-IF. ENA-6 and MPBI had higher negative predictive values than ANA-IF. Two step reflex testing showed significantly higher sensitivity and negative predictive value for all ANA titer positive cases compared to ANA titer ≥1:80, but the specificity was greatly reduced. Part II - MPBI had a higher classification rate for SARDs subtypes than ENA-6.

Conclusions

A one step method (ENA-6 or MPBI) could replace ANA-IF in screening for SARDs and help rule-out other non-SARDs autoimmune conditions. Sequential testing results in a lower sensitivity and negative predictive value in screening for SARDs. MPBI performs better at subtyping SARDs than ENA-6. Finally, automation should help to significantly improve laboratory efficiency.

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS STRAIN USA300 IS PREVALENT AMONG HOSPITAL-ONSET CASES IN AN URBAN CANADIAN SETTING

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Background /objectives

USA300 is the predominant "community-associated" methicillin-resistant Staphylococcus aureus (CA-MRSA) strain in the USA, and has spread to Canada, Spain, Germany and other countries worldwide. The epidemiology of CA-MRSA has recently changed and USA300 appears to be emerging as a cause of serious, hospital-onset infections in large American cities. The aim of this study was to determine if transmission of USA300 MRSA was occurring within two Canadian hospitals.

Methods

All new MRSA cases detected by a hospital-based laboratory over a 12-month period in 2008/2009 were included in the study. Microbiological specimens submitted for testing included both surveillance and clinical specimens. Chart review was performed and cases were defined as hospital-onset MRSA (HO-MRSA) if positive specimens were collected more than 3 days after admission. Bacterial isolates from these cases underwent PCR testing for the Panton-Valentine leukocidin (PVL) gene. All HO-MRSA isolates that were PVL positive underwent pulsed field gel electrophoresis (PFGE) fingerprinting.

Results

In total, 841 new MRSA cases were detected over the study period. Of these,126 were HO-MRSA isolates and underwent testing for PVL. Overall, 39 of 126 (30%) HO-MRSA isolates were found to be PVL positive. All 39 PVL positive isolates that underwent genetic fingerprinting were found to be the USA300 PFGE type.

Conclusions

Our findings indicate that transmission of HO-MRSA USA300 amongst hospitalized patients is common in two Canadian hospitals. This study represents the first known documented report of extensive transmission of HO-MRSA USA300 in Canada. Further, our findings indicate that "community-associated" MRSA may not be an appropriate classification when molecular-based definitions of MRSA are available.

ORAL PRESENTATION

A PROSPECTIVE LONGITUDINAL, STUDY OF THE HUMORAL IMMUNE RESPONSES IN WOMEN INFECTED WITH ONCOGENIC HPV STRAIN(S) TO THE CERVIX USING PROTEIN MICROARRAYS

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Background /objectives

The human papilloma virus (HPV) is one of the most prevalent and commonly transmitted human viruses with more than 200 different known strains in existence. In North America it is estimated that almost every women will be infected at least once in her lifetime with the HPV virus. Although most HPV strains are fairly harmless there are at least 15 virulent HPV strains that are considered to have oncogenic potential and thus have been associated with anogenital cancer. Each oncogenic HPV strain shed 8 proteins of which two have direct oncogenic properties. We set out to build a protein microarray measuring the potential humoral immune response to all the 120 proteins shed by these 15 oncogenic HPV strains. Early detection of antibodies to the HPV proteome could offer possible help in identifying women at risk for progression to anogenital cancer. Also, it could aid in further understanding the complex interaction between the immune system and cancer. The prospective serum samples will come from thousands of volunteering women in British Columbia.

Methods

The samples will be tested on a programmable multiplexed serologic assay called NAPPA (nucleic acid programmable protein array). This assay uses template cDNA encoding for each specific HPV protein. The cDNA is adhered to the microarray and undergoes in situ transcription and translation. Each cDNA is being built according to the sequences found on Papillomavirus Episteme, a NIH database. Each sequence is codon optimized by Block Design. The cDNA coding for each HPV protein is built letting all the primers form templates in a PCA reaction then amplify the templates desired in a PCR reaction. The cDNA building is split up in to building blocks. Each block is inserted in an expression vector and transformed in E.Coli cells by either p-Jet cloning of Topo cloning. Once each block is verified to have the correct sequence they are annealed in order to yield the complete cDNA sequence desired.

Results

The cDNA encoding proteins E6 and E7 from HPV strains 45 and 33 have thus far been built.

THE ROLE OF ICAM-1 ACTIVATION IN MYOCARDIAL DYSFUNCTION DURING SEPSIS

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Background /objectives

Myocardial inflammation occurs in various types of cardiovascular diseases including ischemia reperfusion injury, myocarditis and during sepsis induced myocardial dysfunction. Lipopolysaccharides (LPS) injection increases intercellular adhesion molecule-1 (ICAM-1) expression in cardiomyocyte. We tested the hypothesis that signaling via ICAM-1 contributes to decreased cardiomyocyte and left ventricular contractility in an animal model of systemic inflammation.

Methods

Cardiomyocyte and Left ventricular contractility were measured following injection of LPS in in-vitro and in-vivo experiments

Results

Cardiomyocyte activation increases ICAM-1 expression. ICAM-1 binding decreases cardiomyocyte fractional shortening (reduced by 36 ± 3% from control, $P < 0.01$) by higher expression of focal adhesion kinase. LPS injection significantly increased cardiac ICAM-1 expression and decreased in-vivo measures of left ventricular contractility (end-systolic elastance, Ees decreased 58 +/- 4%, $p < 0.05$, [dP/dtmax]/EDV decreased 60 +/- 6%, $p < 0.05$). In ICAM-1 knock-out mice LPS did not decrease any measure of contractility.

Conclusions

Signaling via cardiac ICAM-1 is necessary to mediate leukocyte-dependent decrease of left ventricular contractility in endotoxemic mice.

SALMONELLA-MEDIATED NEUTROPHIL RECRUITMENT IN MURINE COLITIS ALTERS THE HOST INTESTINAL MICROBIOTA VIA NEUTROPHIL ELASTASE ACTIVITY

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Background /objectives

Gastrointestinal (GI) infections involve an interactive tripartite relationship between the invading pathogen, the host and the host's resident intestinal microbiota. GI pathogens were previously shown to exploit host-mediated inflammation during infection progression. We sought to characterize the inflammatory response and associated microbiota alterations during murine intestinal salmonellosis. Our objective was to determine the pathogen and host factors involved in the establishment of S. Typhimurium in the gastrointestinal tract.

Methods

B57BL/6 mice were pre-treated with a low dose of streptomycin and then infected with S. Typhimurium strains, including mutants in the two Type III secretion systems, Salmonella Pathogenicity Island (SPI) -1 and SPI-2. To characterize the role of neutrophil elastase in this process, mice were treated with either an intraperitoneal (ip) injection of neutrophil elastase inhibitor (Sivelestat sodium salt hydrate) or given oral and ip recombinant neutrophil elastase. Perturbations in the microbial community during infection were assessed via evaluation of total microbial numbers, the proportion of Gamma-Proteobacteria, Terminal Restriction Fragment Lengths Polymorphism (tRFLP) and 16S rDNA pyrosequencing analyses.

Results

Infection with either the wild-type (WT) or SPI-1 mutant, but not SPI-2 mutant strain resulted in significant intestinal inflammation. However, the post-infectious microbiota communities were more similar between mice infected with mutants, rather than the WT strain. Large amounts of neutrophils were observed in the ceca of WT, but not mutant-infected mice, implicating neutrophils as a mediator of microbiota perturbations observed in WT salmonellosis. Inhibition of neutrophil elastase activity in Salmonella-infected animals prevented the induction of microbiota alterations observed in animals with an intact immune response. Conversely, administration of recombinant neutrophil elastase to infected animals under conditions that do not elicit neutrophil recruitment, caused shifts in microbiota composition that favored Salmonella colonization.

Conclusions

These studies underscore the importance of host immune response in inducing the alterations in intestinal microbiota that favor pathogen colonization during infection. We identify neutrophils as a central mediator of these perturbations and demonstrate that neutrophil elastase activity in the gut facilitates Salmonella colonization.

IMPAIRED WOUND HEALING IN APOLIPOPROTEIN E DEFICIENT MICE

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Background /objectives

The purpose of this study is to establish the apolipoprotein E (ApoE) knockout (KO) mouse as a model of impaired wound healing. ApoE functions as a key mediator of circulating cholesterol and deficiencies in ApoE can result in hypercholesterolemia. In addition, ApoE is highly expressed in the skin, where it can regulate inflammation through its anti-oxidative and anti-inflammatory properties. Mice deficient in ApoE develop an inflammatory skin phenotype prone to accelerated aging featuring increased thinning, collagen disorganization and susceptibility to injury. We hypothesized that ApoE KO mice will demonstrate increased inflammation in response to injury thereby impairing the wound healing process.

Methods

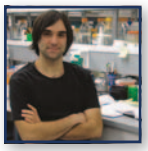
Seven week old C57BL/6 wild type mice and apoE KO mice were given a single 1 cm diameter full thickness skin wound on their mid backs. Wound tissue was harvested at 2, 8 or 16 days post-wounding and analyzed histologically.

Results

Wound closure times for all groups of mice were similar, with wounds showing full closure by 16 days. Histological analysis of the newly formed dermis revealed an increase in inflammatory cells in the ApoE KO mice, as well as alterations in collagen structure. While healed tissue from wild type mice demonstrated the formation of new sebatious glands, hair follicles and mature collagen, these were not observed in ApoE KO mice.

Conclusions

Wound healing in ApoE KO mice is impaired compared to wild type controls, featuring increased inflammation and altered collagen/dermal structure.



GRANZYME B CLEAVAGE OF FIBRONECTIN LEADS TO REDUCED ENDOTHELIAL CELL ADHESION MIGRATION AND CAPILLARY TUBE FORMATION

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Background /objectives

Immune-derived proteases regulate neovascularisation in part through cleavage of extracellular matrix (ECM) proteins that interact with infiltrating endothelial cells (EC) on their way to form functional capillaries. Granzyme B (GZMB) is a serine protease that is expressed and released by a variety of immune cells and accumulates in the ECM during chronic inflammatory diseases that are highly associated with dysregulated angiogenesis. Although several ECM proteins have been identified as GZMB substrates, the effect of GZMB-mediated ECM cleavage on EC angiogenic potential has not been determined. Fibronectin (FN) is an important ECM protein that regulates angiogenesis by facilitating EC adhesion and migration. We hypothesized that GZMB cleavage of FN will disrupt EC angiogenic behaviour due to degradation of FN.

Methods

GZMB cleavage of FN, both in its plasma form and matrix form, was evaluated by western blot. Human microvascular endothelial cells (HMVEC) adhesion and migration to FN treated with either GZMB or GZMB inhibitor was evaluated in FN coated culture wells and modified Boyden chamber assay. Capillary tube formation assay in collagen gel mixed with FN was performed in order to examine EC capacity to form capillary tube structures in the presence of GZMB.

Results

GZMB effectively and dose dependently cleaved human plasma FN, both in its soluble form and matrix form. GZMB-mediated FN cleavage resulted in a significant reduction in HMVEC adhesion and migration to fibronectin, which was restored by GZMB inhibition. GZMB treatment reduced EC capacity to form capillary tube structure in collagen gel matrix.

Conclusions

GZMB cleavage of FN disrupts EC angiogenic behaviour and prevents capillary. GZMB may contribute to impaired angiogenesis during chronic inflammation while inhibition of GZMB activity can prevent FN degradation and may support physiological angiogenesis

A RADICAL NEW THERAPY FOR MULTIPLE SCLEROSIS? IMMUNOMODULATORY AND NEUROPROTECTIVE PROPERTIES OF THE ANTIOXIDANT TEMPOL

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

Background /objectives

Multiple sclerosis (MS) is one of the most common and most debilitating neurological diseases of young adults in Canada, with Canadians having one of the highest rates of MS in the world. Reactive oxygen and nitrogen species are implicated in inflammation-mediated damage to the central nervous system (CNS) in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). We have shown that oral administration of the antioxidant TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), a stable nitroxide radical, lowers incidence and reduces the severity of symptoms in EAE. We hypothesize that TEMPOL limits inflammatory demyelinating disease by regulating the development of pathogenic autoimmune responses and limiting alterations to the blood-brain barrier (BBB). Our studies aim to identify the mechanisms by which TEMPOL reduces damage and assess its therapeutic potential in MS.

Methods

Immune responses and tissue inflammatory infiltrates were compared between control and TEMPOL-fed EAE mice. Ex vivo analysis compared the phenotype of autoreactive cells from lymphoid organs and the brain using cytokine measurement by ELISA and flow cytometry. Brain microvessel endothelial cells were used as an in vitro model to characterize the influence of TEMPOL on BBB integrity. Permeability was assessed using fluorescently-labeled macromolecules in diffusion assays and transendothelial electrical resistance measurements. BBB cell adhesion molecule expression under inflammatory conditions was characterized in vitro by ELISA.

Results

Autoreactive T cell responses were comparable in magnitude, yet TEMPOL-fed animals produced less transforming growth factor (TGF)-beta and less of the proinflammatory cytokine interferon (IFN)-gamma in response to a variety of stimuli. Flow cytometry revealed fewer immune infiltrates in the CNS of TEMPOL-fed animals, which correlated with less severe disease. In the periphery, we observed enrichment of CD8 over CD4 T cells in lymphoid tissues of TEMPOL-fed EAE mice compared to controls. TEMPOL treatment enhanced BBB integrity at rest and limited changes in transendothelial resistance induced by the inflammatory mediators tumor necrosis factor (TNF)-alpha and interleukin (IL)-1 beta. TEMPOL also limited cytokine-mediated upregulation of cell adhesion molecules, proteins that mediate immune cell recruitment across the BBB.

Conclusions

Our results illustrate the ability of TEMPOL to regulate key pathological processes in the development of chronic CNS inflammation and demyelinating disease. TEMPOL alters the phenotype of autoreactive T cells and limits BBB alterations, including the potential for immune cell entry to the CNS. Taken together, these immunomodulatory and neuroprotective properties demonstrate TEMPOL's potential as a novel and efficacious therapeutic in MS.

TREGS FROM T1D SUBJECTS WITH A SUSCEPTIBLE IL-2R GENE SNP, RESPOND ABERRANTLY TO IL-2 STIMULATION

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Background /objectives

Type 1 diabetes (T1D) is an autoimmune disease resulting from the destruction of insulin-producing beta cells by autoreactive lymphocytes. Regulatory T cells (Tregs) maintain self-tolerance in part by suppressing the effector functions of self-reactive lymphocytes. Treg dysfunction may therefore be a key factor in the pathogenesis of autoimmune diseases such as T1D. Tregs require the cytokine interleukin-2 (IL-2) for survival and maintenance of their suppressive function. IL-2 binds to the high-affinity IL-2 receptor (IL-2R), leading to phosphorylation of signal transducer and activator of transcription 5 (STAT5) and downstream transcription of effector proteins. Activation of the STAT5 signaling pathway is required to maintain high expression of the transcription factor FOXP3, which sustains the suppressive function of Tregs. The IL-2 receptor gene has single nucleotide polymorphisms (SNPs), which are strongly associated with T1D incidence. We hypothesized that T1D subjects with SNPs in the IL-2R gene possess Tregs that respond aberrantly to IL-2 and therefore do not optimally induce signaling in the STAT5 pathway. Our results indicate that Tregs from individuals with the homozygous susceptible GG genotype have reduced levels of pSTAT5 compared to those with the AA genotype (p=0.0133). This provides evidence that Tregs from T1D subjects with SNPs in the rs3118470 locus of the IL-2R gene have diminished STAT5 phosphorylation in response to IL-2. This is a potential mechanism of defective Treg function in a genetically identifiable subset of children with T1D.

Methods

PBMC from T1D subjects with a homozygous susceptible (GG), heterozygous (AG) and homozygous wild-type (AA) genotype for a SNP located in the rs3118470 locus of the IL-2R gene were stimulated with IL-2, prior to fixation and staining for CD4, FOXP3, CD25 and pSTAT5.

Results

Our results indicate that Tregs from individuals with the homozygous susceptible GG genotype have reduced levels of pSTAT5 compared to those with the AA genotype (p=0.0133).

Conclusions

This provides evidence that Tregs from T1D subjects with SNPs in the rs3118470 locus of the IL-2R gene have diminished STAT5 phosphorylation in response to IL-2. This is a potential mechanism of defective Treg function in a genetically identifiable subset of children with T1D.

NATURAL KILLER T CELLS CONTROL B CELLS INFECTED AND TRANSFORMED BY EPSTEIN-BARR VIRUS

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Background /objectives

Natural killer T (NKT) cells are innate-like lymphocytes that recognize lipid antigens presented by the evolutionarily conserved molecule, CD1d. Individuals lacking NKT cells, such as patients with X-linked lymphoproliferative disease, develop severe and often fatal lymphoproliferative disorders following Epstein-Barr virus (EBV) infection. Our overall hypothesis is that NKT cells are critical for generating optimal responses to EBV and that the absence of NKT cells in patients with XLP leads to poor immune activation following EBV infection. The objective of this study was to assess whether NKT cells are capable of regulating the transformation of B cells by EBV.

Methods

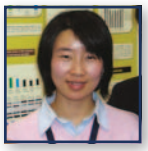
To determine whether NKT cells control EBV-infected target cells, we established an in vitro experimental system using human NKT cell lines and EBV-transformed tonsillar B cells (lymphoblastoid cell lines [LCL]). NKT cell lines were co-cultured with LCL generated from tonsils of healthy individuals and NKT cell activation was measured by the production of IFN-gamma (ELISA) and using standard chromium release cytotoxicity assays.

Results

EBV transformation of naïve B cells into lymphoblastoid cell lines LCL resulted in the downregulation of CD1d but not the conventional peptide antigen presentation molecules major histocompatibility complex (MHC) class I and MHC class II. In contrast to naïve B cells, LCL loaded with the exogenous NKT cell agonist alpha-galactosylceramide (alpha-GC) failed to activate IFN-gamma secretion or cytotoxicity by NKT cell lines due to the loss of CD1d. To determine whether CD1d expression on LCL could stimulate NKT cell recognition, EBV-transformed B cells were incubated with the synthetic retinoic acid receptor-alpha (RAR-alpha) agonist AM580, which upregulates the expression of CD1d. AM580 elevated the transcriptional and surface expression of CD1d and unexpectedly rendered LCL, but not AM580-treated naïve B cells, capable of activating robust NKT cell IFN-gamma secretion and cytotoxicity even in the absence of alpha-GC. Effector functions of NKT cell lines were further enhanced when AM580-treated LCL were pulsed with alpha-GC.

Conclusions

Our results imply that EBV-transformed B cells evade NKT cell surveillance in vivo by eliminating CD1d expression and suggest that RAR-alpha agonists such as AM580 and may have therapeutic use for augmenting NKT cell functions.



MICRORNA(MIR)-126 PROMOTES COXSACKIEVIRUS B3 INDUCED CELL DEATH BY TARGETING WNT SIGNALING PATHWAY

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Background /objectives

Viral myocarditis is an infectious heart disease mainly threatening children and young adults. It is commonly caused by coxsackievirus B3 (CVB3) infection which leads to inflammatory infiltration of mononuclear cells into myocardium resulting in cardiomyocyte damage or death. The virus-induced cell death is also essential for viral progeny release that allows the virus to infect surrounding healthy tissues. A better understanding of the mechanism by which CVB3 regulates the cell death would be the key factor for developing effective treatment for this disease. MicroRNAs(miRNAs) are recently indentified small endogenous gene regulators. They regulate their target gene expression at the post-transcriptional level in a similar manner as small interfering RNAs (siRNAs). They are involved in various physiological and pathological processes including infectious diseases. Our previous studies showed that miR-126, one miRNA abundant in the heart enhanced CVB3 replication by regulating ERK signaling. Here we further investigated the effect of miR-126 on the CVB3 induced cell apoptosis and viral progeny release.

Methods

We ectopically expressed miR-126 or scrambled control miRNA in HeLa cells by transfection and then infected the cells with CVB3 at 10 MOI for 0-8h. The virus-induced cell death was measured by morphology observation under a microscope, MTS cell viability assay, and Western blot (WB) analysis of pro-caspase-3 cleavage. The replication of CVB3 was evaluated by WB to detect viral protein and viral plaque assay to measure viral titer. The target genes of miR-126 were predicted by TargetScan, a bioinformatic tool, and validated using WB and luciferase assay. The role of the miR-126 targeted genes in CVB3 induced cell death was investigated by introducing specific siRNAs against these genes into HeLa cells and detecting cell apoptosis and viral release.

Results

i) Ectopic expression of miR-126 enhancedCVB3-induded cell morphological changes, which are characterized by cell rounding and detaching. This typical cell apoptosis was verified by increased pro-caspase-3 cleavage and confirmed by MTS assay showing decreased cell survival in miR-126 group as compared to the control group. ii) The viral capsid protein 1 (VP1) synthesis was enhanced by miR-126. The release of the viral particles was increased more than 100 times in the miR-126 group. iii) Both bioinformatic prediction and luciferase assay indentified that WRCH1 and LRP6, both of which are involved in Wnt signaling, are two novel specific targets of miR-126. iv) Ectopic expression of miR-126 inhibited the Wnt signaling pathway activity as determined by WB analysis of phospho-GSK3-beta, degradation of beta-catenin and Wnt lusciferase assay. Finally, v) Silencing of WRCH1 and LRP6 by specific siRNAs showed similar effect as miR-126 in suppressing Wnt signaling, enhancing cell death and promoting CVB3 progeny release.

Conclusions

Signaling via cardiac ICAM-1 is necessary to mediate leukocyte-dependent decrease of left ventricular contractility in endotoxemic mice.

MEASURING MITOCHONDRIAL DNA OXIDATIVE DAMAGE BY HIV NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS IN CULTURED CELL MODELS

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Background /objectives

There are 34 million people currently living with HIV worldwide. Treatment with lifelong highly active antiretroviral therapy (HAART) has greatly decreased mortality and morbidity in HIV+ individuals. Nucleoside reverse transcriptase inhibitors (NRTIs) form the backbone of HAART and have been implicated in mitochondrial dysfunction. Aging and age-associated diseases such as cardiovascular disease and cancer are reported to occur earlier in the HIV infected population. While the mechanisms for this is not fully understood, it is possibly linked to mitochondrial dysfunction. We hypothesize that NRTIs may contribute to mitochondrial toxicity by increasing oxidative damage to mitochondrial DNA (mtDNA). A qPCR-based assay that quantifies mtDNA oxidative damage by exploiting the fact that oxidative damage slows down the progression of PCR polymerase was optimized. Using this assay, the amplification of study mtDNA relative to an undamaged control mtDNA is used to quantify relative levels of mtDNA oxidative damage. We measured mtDNA oxidative damage in cultured human cells exposed to NRTIs.

Methods

Human coronary artery endothelial (hCAE) cells were exposed to the following NRTIs, TDF, 3TC, AZT, and d4T, for a period of ~30 days at clinically relevant concentrations. Human colorectal adenocarcinoma (HT29) cells were exposed to the following NRTIs, TDF, 3TC, AZT, d4T, ddI, and ABC at various concentrations (with the exception of 3TC) for a period of ~30 days. Cells were collected longitudinally at various time points and DNA was extracted. The DNA was then quantified by mtDNA copy number, amplified by long template PCR and products of this PCR were quantified also by qPCR mtDNA copy number. The mtDNA oxidative damage of NRTI-exposed cells was expressed relative to that of unexposed control cells. The longitudinal mtDNA content was also quantified using qPCR.

Results

In hCAE cells, mtDNA oxidative damage was detected with d4T and TDF exposure but not 3TC or AZT. No mtDNA oxidative damage was detected with any of the NRTIs in the HT29 culture. Depletion of mtDNA content was demonstrated in both cultures with d4T exposure, and in a dose dependent manner with HT29 cells, along with ddI. No changes were seen in mtDNA content with the other NRTIs in both cultures.

Conclusions

Despite lower NRTI concentrations used, greater mtDNA oxidative damage was seen with primary hCAE cells, compared to immortalized HT29 cells. Of particular interest was evidence of mtDNA oxidative damage seen with TDF which is currently recommended in first line HAART. Differences seen in mtDNA oxidative damage in the two cultures suggest that the two cell lines are affected differently by NRTI exposure despite the fact that drugs such as d4T are active in both as reflected by the observed decrease in mtDNA content. For example, HT29 cells may have undergone adaptations that protect them in unfavorable conditions conditions such as oxidative stress. Primary cells such as hCAE cells may therefore offer a more appropriate model for the study of NRTI-induced toxicity including mtDNA oxidative damage.

FACTORS ASSOCIATED WITH SHORTER LEUKOCYTE TELOMERE LENGTH IN HIV+ AND HIV- ADULTS

DeAnna Zanet[1], Anona Thorne[3], Beheroze Sattha[1], Evelyn J Maan[2], Izabelle Gadawski[1], Melanie Murray[1,2,4], Joel Singer[1,3], Deborah M Money[1,2,4], Neora Pick[1,2], Helene CF Côté[1,4] and the CIHR Emerging Team in HIV Therapy and Aging (CARMA)

[1]University of British Columbia; [2]Oak Tree Clinic; [3]CIHR Canadian HIV Trials Network; [4]Women's Health Research Institute

Background /objectives

Telomeres protect the ends of chromosomes and shorten with each cell division in somatic cells, acting as a marker for aging. In germ cells and stem cells, including hematopoietic and tissue stem cells, telomerase is expressed and telomere length (TL) is maintained. Telomerase has reverse transcriptase (RT) activity and is homologous to HIVRT. Nucleoside reverse transcriptase inhibitors (NRTIs) given as part of highly active antiretroviral therapy (HAART) can inhibit telomerase which can lead to telomere shortening. Oxidative stress due to HIV-induced immune activation and/or chronic inflammation may also shorten telomeres. We investigated predictors of shorter leukocyte telomere length (LTL) in HIV+ and HIV- adults.

Methods

HIV+ and HIV- adults aged 19-75 were prospectively enrolled in the CARMA cohort on HIV therapy and aging. Whole blood relative average LTL was measured using qPCR. Univariate linear regressions were used to examine the relationships between potential explanatory variables and LTL. Univariately important variables ($p<0.15$) were used to develop multivariate linear regression models. Interactions and associations between variables were also explored.

Results

Data was obtained for 229 HIV+ subjects (40.3 ± 10.5 years, 79% female) and 166 HIV- controls (39.3 ± 11.1 years, 71% female). Shorter LTL were univariately associated with older age, younger paternal and maternal ages at birth, smoking, illicit drug use, hepatitis C virus (HCV) diagnosis ever, HIV+ status, non-Black/South Asian ethnicity, and income <$15K/year. Parental ages, although important, were not included in the multivariate model as data was missing for 210/395 subjects. Several explanatory variables were correlated, including: drug use/ethnicity/HCV and income/smoking, potentially confounding each other. There were also significant interactions whereby smoking, illicit drug use, HCV and income were strongly associated with shorter LTL in the HIV- group, and associated less so or not at all in the HIV+ group, increasing modeling complexity. Age ($p<0.0001$), HIV+ status ($p=0.034$), HCV ($p=0.005$), low income ($p=0.07$) and pack*years smoking ($p=0.099$) were associated with shorter LTL in a model adjusted for interactions ($R2=0.26$). Among HIV+ subjects, HIV duration, antiretroviral therapy duration, current or nadir CD4, and HIV plasma viral load showed no association with LTL.

Conclusions

These results suggest that in addition to aging, HIV+ status, HCV infection (or drug use) low income and smoking are the strongest predictors of LTL and may therefore affect telomere maintenance and cellular aging. None of the HIV-specific factors tested showed any association with LTL, suggesting that viral infections and lifestyle factors may exert greater influence on telomere maintenance. Future work should attempt to further elucidate these factors and determine whether active vs. cleared HCV vs. drug use is most closely linked to LTL.

INHIBITION OF CASTRATION RESISTANT PROSTATE CANCER BY SINTOKAMIDE A-A NOVEL ANTAGONIST OF THE AMINO-TERMINUS OF THE ANDROGEN RECEPTOR

Iran Tavakoli[1], Nasrin R Mawji[1], Jun Wang[1], Luping Yan[2], David E Williams[2], Raymond J Andersen[2], and Marianne D Sadar[1]

[1]Department of Genome Sciences Centre, BC Cancer Agency; [2]Chemistry and Earth & Ocean Sciences, University of British Columbia

Background /objectives

Lethal castration resistant prostate cancer (CRPC) is considered to involve a transcriptionally active androgen receptor (AR). All current therapies target AR ligand-binding domain (LBD) to inhibit receptor activity. Unfortunately these therapies fail presumably by mechanisms involving breakthrough of androgen synthesis and/or expression of constitutively active AR splice variants that lack LBD. Recent studies have provided proof-of-concept that AR N-terminus domain (NTD) is druggable with the development of EPI-001, an antagonist that has specificity and efficacy on both androgen-sensitive and CRPC xenografts. Here we reveal an unrelated class of compounds called SINT1 as a potent therapeutic agent for CRPC that targets AR NTD.

Methods

Candidate compounds were identified by high throughput screening methods. The potency of compounds were demonstrated by effects on AR regulated transcriptional activity and gene expression, steroid receptor specificity, cell proliferation, and CRPC xenografts. Materials & Method: To examine the effects of SINT1 on AR transcriptional activity, both endogenous gene expression and reporter gene constructs that are regulated by AR were examined. The ability of SINT1 to directly inhibit transactivation of the AR NTD was tested using a chimera of AR NTD fused to the DNA-binding domain of Gal4. Reporter assays for glucocorticoid (GR) and progesterone receptor (PR) were measured to ensure specificity. BrdU incorporation was analyzed to indicate changes in proliferation of AR positive and negative cell lines. Animals bearing LNCaP subcutaneous xenografts were castrated when tumours were 100 mm3 and randomized into two groups. One week after castration, the animals were treated every 3 days with an intratumoral dose of SINT1(30 mg/kg) or vehicle.

Results

SINT1 blocked AR activity as measured by reduction in PSA mRNA and reporter activity induced by androgen. SINT1 reduced transactivation of AR NTD to baseline levels. SINT1 had no effects on transcriptional activities of related steroid receptors, GR and PR. SINT1 was effective in blocking androgen-induced proliferation in LNCaP cells but did not decrease proliferation of PC3 cells that lack functional AR. Importantly, some SINT1-treated CRPC xenografts regressed while others did not grow (mean = 103.3± 11.97 mm3, n=7) with no change in animal body weight compared to DMSO-treated tumors (n=11) that grew to 153.7± 11.87 mm3 (p=0.011).

Conclusions

Together these data support that SINT1 is a specific inhibitor of the AR NTD without effects on highly related steroid hormone receptors and no apparent toxicity in animals. SINT1 is an antagonist to AR NTD that causes regression of CRPC xenografts.

Y BOX PROTEIN 1: A NOVEL DIRECT TRANSLATIONAL ACTIVATOR OF HYPOXIA INDUCIBLE FACTOR 1α STEERING SARCOMA CELL INVASION & METASTATIC DISSEMINATION

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Background /objectives

To identify the potential roles played by YB-1 in sarcomas progresstion. Aim: 1- Identify the effect of YB-1 knockdown on the invasive capacity and metastatic behavior of sarcoma cell lines in vitro and in vivo. 2- Identify the contribution of YB-1 to the hypoxic response and to HIF1α translational regulation.

It is estimated that distant tumor metastases are responsible for over ninety percent of cancer deaths. Of malignancies frequently displaying the metastatic phenotypes are sarcomas. Sarcomas are aggressive groups of malignancies of mesenchymal origin commonly affecting pediatric age groups of which being characterized by early metastatic spread and poor prognosis. Intratumoral hypoxia represents a unifying characteristic feature of tumor microenvironment in solid malignancies including sarcomas, and through the central transcriptional response factor for hypoxic adaptation; HIF1α, can markedly influence the metastatic potentiality of tumor cells. Besides activating HIF1α and its signaling pathways during hypoxia, reprogramming of the translational machinery takes place with inhibition of cap-dependent translation and activation of IRES-dependent translation of certain mRNA whose function to support survival, and promote escape of tumor cells through angiogenesis as well as inhibit apoptosis. YB-1, a member of evolutionary conserved CSD family of proteins functions in regulating DNA transcription and RNA processing/translation, is a general suppressor of cap-dependent translation under various stress conditions. Our group recently demonstrated that YB-1 over-expression induces an epithelial-to-mesenchymal transition (EMT) in H-Ras transformed human mammary epithelial MCF10A cells and enhances their metastatic capacity at the expense of cell growth through IRES-dependent (Cap-independent) translational activation of EMT-associated messages including HIF1α. However, the potential contribution of YB-1 to the hypoxic response and how this may impact sarcoma cells metastatic ability have remained totally unknown. Hypothesis: Elevated YB-1 levels, observed in sarcomas, promote the invasion of sarcoma cells and contribute to their aggressive phenotype. More specifically, YB-1 alters the activity of the downstream effector hypoxia-inducible factor 1 alpha (HIF1α) to promote the metastatic phenotype.

Methods

MNNG&MG63 (osteosarcoma), TC32&TC71 (Ewing Sarcoma), and Rh30 (rhabdomyosarcoma) cell lines were chosen as models for the current study. Transient and stable YB-1 knockdown cells were created and assessed with different assays. Cell motility was assessed in vitro by wound healing and Boyden chamber assays. Hypoxia chamber incubations immunoblotting, Polysomal fractionation and real-time RT-PCR were used to study the relationship between YB-1 and HIF1α and their ability to promote metastasis. NOD/SCID mice and zebrafish animal models were used to study the impact of YB-1 downregulation on tumor cell metastatic behavior in vivo.

Results

We found that under hypoxic conditions, both YB-1 and HIF1α were induced. Inhibiting YB-1 expression has led to a corresponding decrease in HIF1α expression under hypoxia which was also associated with enhanced apoptotic cell death. Using Polysomal fractionation and qPCR, we determined that YB-1 is a major translational regulator of HIF1α. Interestingly, in vivo results clearly demonstrated that YB-1 downregulation is associated with inhibition of tumor cell metastatic capacity. These findings may provide a mechanism to help explain how YB-1 promotes sarcoma cell metastasis.

CHARACTERIZATION OF ANDROGEN RECEPTOR-TARGETING SMALL MOLECULE TERPENES FOR PROSTATE CANCER

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Background /objectives

The incidence and mortality rates of prostate cancer (PCa) remain high among North American men, and men with recurrence or advanced PCa are incurable. Androgen deprivation therapy or castration, the mainstay treatment for the advanced disease, can only provide palliative effect before the patients eventually develop lethal castration-resistance prostate cancer (CRPC). Androgen receptor (AR), a ligand-activated transcription factor, plays a critical role in CRPC. Aberrant AR activity is frequently detected in both animal models and clinical samples. Therefore, AR-targeted therapies possess significant potential to treat advanced PCa and CRPC. The objective of this study is to provide preclinical characterization of small molecule Terpenes (T1, T2, and T3) by evaluating their in vitro and in vivo AR antagonistic efficacies.

Methods

Human PCa cell lines were used to evaluate the effects of Terpenes on cellular proliferation by quantifying the rate of DNA synthesis. To measure AR transcriptional activity AR-driven reporters were transfected into LNCaP cells, which were subsequently treated with Terpenes. Cells were harvested 48 hours later to measure the reporter activity. To confirm AR inhibition by Terpenes, the endogenous expression levels of several known AR-regulated genes were measured by qRT-PCR. To demonstrate steroid receptor specificity, the effect of Terpenes on the transcriptional activity of glucocorticoid receptor (GR) was measured. Furthermore, in vitro ligand-binding competition assay was employed to confirm the binding of Terpenes to AR. Finally, male intact mice were treated with Terpenes to provide preliminary in vivo efficacy.

Results

Structurally related Terpene compounds displayed distinct functional activities. While T2 remained inactive or showed weak activity, both T1 and T3: (1) inhibited androgen-dependent proliferation in human PCa cells; (2) blocked androgen-induced AR transcriptional activity; (3) reduced endogenous expression of AR-regulated genes; (4) demonstrated receptor specificity by showing no effect on GR transcriptional activity; and (5) bound directly to the ligand-binding domain of AR with strong affinities. From the in vitro ligand-binding assay, T3 exhibited the highest binding affinity to AR among the Terpenes, and was therefore selected for preliminary animal testing in NOD SCID male intact mice. Daily treatment of T3 for 2 weeks significantly reduced (~20% smaller compared to the no-treat control) the size of seminal vesicle, which is an androgen-dependent tissue. Notably, mice treated with T3 did not show significant changes in weight, suggesting that T3 had no apparent adverse effects.

Conclusions

Among the Terpenes, T3 was characterized to be a potent in vitro AR antagonist by directly binding to AR and inhibiting AR-dependent transcription and cellular proliferation. Preliminary studies in mice showed that T3 significantly decreased androgen-dependent tissue such as the seminal vesicle, confirming T3's in vivo efficacy. Together these data suggest that further evaluation of Terpenes could contribute to the discovery of effective treatments for CRPC.

THE ROLE OF HYPOXIA INDUCED SECRETED PROTEINS IN BREAST CANCER METASTASIS

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Background /objectives

Metastatic tumours are responsible for 90% of cancer related deaths. However, most research has focused on the primary tumour, while the process of metastasis is poorly understood. Recently, the pre-metastatic niche hypothesis to explain how metastasis occurs has gained acceptance. The pre-metastatic niche represents an area in metastatic target organs where the extra cellular matrix has been remodeled by tumour secreted proteins and there has been an accumulation of bone marrow derived cells. There is evidence that these bone marrow cells are immature myeloid cells and myeloid derived suppressor cells (MDSC's). Together the architectural change in extra cellular matrix and the presence of immunosuppressive myeloid cells creates an area permissive to the invasion, survival and growth of circulating tumour cells. It is known that poorly oxygenated (hypoxic) tumours have a more aggressive and invasive phenotype which correlates with a negative prognosis. Functionally limited tumour blood vessels cause a fraction of the tumour to become hypoxic and express hypoxia inducible proteins. These proteins are regulated through hypoxia inducible factor (HIF) transcription factors and are responsible for increased angiogenesis, invasion, matrix deposition and remodeling. We hypothesize that hypoxic tumours produce and secrete proteins distinct or at increased levels compared to normoxic tumours and that those secreted proteins are required for pre-metastatic niche development. Our aim is to identify the hypoxia induced secreted protein(s) responsible for the mobilization and accumulation of MDSC's in metastatic target organs.

Methods

We utilized Stable Isotope Labeling of Amino acids in Cell culture (SILAC) to perform a quantitative proteomic screen of conditioned media. Mammary carcinoma cells derived from a Balb/c mouse (4T1 tumour cell line) were incubated in 1% O2 (hypoxic) or 20% O2 (normoxic) for 24 hours. The secreted proteins were collected and subjected to mass spectrometry (LC/MS-MS). We generated a list of secreted proteins and relative expression levels of hypoxic verses normoxic secreted proteins. In addition, we looked at the ability of hypoxic conditioned media to induce the mobilization and accumulation of myeloid derived suppressor cells (MDSC's) in the lungs and spleen of naive mice after repeated daily intraperitoneal injection.

Results

The proteomic data shows an up regulation of hypoxia inducible proteins as well as proteins associated with pre-metastatic niche development. Tenascin C is a known metastasis associated protein and was chosen as a candidate protein based on a 16 fold increase over the normoxic level of secretion. Injection of conditioned media from 4T1 cells did not yield an increase of MDSC's in lungs or spleen of Balb/c mice. However, the conditioned media did yield a 6 fold increase of MDSC's in the lungs of NOD/SCID mice.

Conclusions

While hypoxia results in an up regulation of metastasis associated proteins, it appears that hypoxia secreted proteins alone are not sufficient to cause MDSC accumulation in metastatic target organs of immunocompetent mice. Tenascin C is a candidate hypoxia induced secreted protein. Using lenti viral transfections, we will knock down and over express Tenascin C to examine the effect on metastasis.

LCAT DEFICIENCY DOES NOT AFFECT BETA-AMYLOID PATHOLOGY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE DESPITE DRAMATICALLY REDUCED CIRCULATING APOA-I LEVELS

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Background /objectives

High-density lipoproteins (HDL), also known as the "good cholesterol", are important for heart and brain health. ApolipoproteinA-I (apoA-I) is the major protein component of peripheral HDL, whereas apolipoproteinE (apoE) serves this function in the central nervous system (CNS). Although apoA-I is not expressed by astrocytes and microglia, which synthesize and secrete apoE, apoA-I is found at relatively high levels in the cerebrospinal fluid (CSF), 0.01-0.5% of plasma levels, and is detectable in brain tissue lysates. Although well known to be associated with an increased risk of cardiovascular disease (CVD), low levels of apoA-I or HDL at mid-life are also associated with an increased risk for development of dementia later in life. In mouse models of Alzheimer's Disease (AD), the most common form of dementia, apoA-I is involved in cognitive function, deposition of amyloid in the cerebral vasculature, and inflammation. Lecithin-cholesterol acyltransferase (LCAT) is an enzyme responsible for esterifying free cholesterol, a key step in generating mature HDL particles. Mutations in human LCAT result in two distinct metabolic diseases, Familiar LCAT Deficiency and Fish Eye Disease, both characterized by low plasma HDL levels and increased risk for premature CVD. However, little is known about how or if LCAT deficiency affects HDL metabolism in the CNS and possible affects on AD pathology.

Methods

15-16 month-old male APP/PS1 and APP/PS1/LCAT-/- mice were used, N=5-6 per group. Following a 4-hour fast, CSF and plasma were collected, animals were perfused, and brain tissue was isolated and either snap frozen or fixed in 3.7% paraformaldehyde. For protein analysis, tissue was serially extracted using carbonate followed by 5M guanidine hydrochloride buffers. Levels of target proteins were determined by denaturing immunoblotting and ELISA. Amyloid deposition in the brain parenchyma and vasculature was determined histologically by thioflavinS staining, using Image Pro software for analysis.

Results

Deletion of LCAT resulted in a significant 71% reductions of apoA-I in plasma ($p<0.001$) and 89% in CSF. However, apoA-I protein levels remain unchanged in the cortex and hippocampus compared to controls. Protein levels of apoE, the cholesterol transporter ABCA1, and low density lipoprotein receptors LDLR and LRP, were unchanged. In addition, no change was observed in levels of Abeta 1-40 or Abeta 1-42 in either the carbonate soluble or insoluble fraction, or in amyloid burden.

Conclusions

These results suggest that the brain may have alternative apoA-I regulatory or uptake mechanisms independent of LCAT function that allow apoA-I protein levels to remain constant despite dramatic reductions in CSF and plasma. Given that levels of key proteins known to be involved in beta-amyloid metabolism, namely ABCA1, apoE, apoA-I, LRP, and LDLR, are unchanged in the brain it is reasonable to conclude that LCAT activity does not affect Alzheimer's pathology in mice. These results are consistent with the lack of overt neurological signs in LCAT deficient patients.

INCREASING INCIDENCE OF MYASTHENIA GRAVIS AMONG ELDERLY IN BRITISH COLUMBIA, CANADA: 1984-2011

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Background /objectives

Myasthenia gravis (MG) is an antibody-mediated autoimmune disease of the neuromuscular junction, characterized by weakness and fatigability of skeletal muscle. The primary antigenic target in MG is the nicotinic acetylcholine receptor (AChR), a ligand-gated sodium channel located at high density on the post-synaptic muscle cell membrane. Anti-AChR antibodies are detected in sera of 80-90% of generalized MG patients and 40-70% of patients with ocular MG. Their high specificity makes anti-AChR seropositivity an excellent surrogate marker for MG. This study aimed to evaluate changes in the incidence and of anti-AChR antibody-seropositive MG in British Columbia (BC), Canada, between the years of 1984 and 2011.

Methods

The Neuroimmunology Laboratory at the University of British Columbia is the sole laboratory in BC that provides anti-AChR antibody testing for clinical diagnosis. Incident cases of anti-AChR seropositivity were identified by retrospectively identifying all first-time cases of anti-AChR antibody-seropositivity in the period of January 1984 to December 2011. Incidence was defined as the annual number of first-time anti-AChR seropositive cases. Incidence rates (IRs) were calculated per 1 million inhabitants based on annual July population estimates (www.bcstats.gov.bc.ca). Cases were stratified into four ages groups based on age at the first positive test: ≤19, 20-44, 45-64 and ≥65 years. Age- and sex-stratified IRs were calculated using population estimates of the corresponding age and sex group.

Results

Between January 1984 and December 2011, 1493 individuals were identified to be anti-AChR antibody-seropositive (767 women, 718 men, 8 unknown). In women the age at the first positive serum sample had a bimodal distribution with peaks at 45-55 and 70-85 years, while in men there was a single peak at 70-80 years. The annual incidence of new AChR seropositivity has increased from 11.0/million (1984-1988 average) to 16.7/million (2007-2011 average). The incidence has most dramatically increased in the elderly (≥65 years), from 21.4/million (1984-1988 average) to 61.9/million (2007-2011 average). In contrast, the mean annual IRs of the three younger age groups has remained relatively constant over the 28-year period. These results confirm that the trend we had described in an earlier report, of increasing incidence of elderly-onset anti-AChR seropositive MG in BC for the period of 1984-2008, is continuing (Pakzad Z et al., Neurology 2011).

Conclusions

Our results indicate a continually increasing incidence of elderly-onset anti-AChR seropositive MG in BC which is not due to demographic changes. This trend could be due to improved diagnosis of MG in elderly patients over the decades, or a true increase in the incidence due to as yet undefined reasons.

REGULATORY T CELLS IN CNS INFLAMMATION

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Background /objectives

Regulatory T cells (Tregs) are thymus-derived CD4+ CD25+T lymphocytes that play an important role in maintenance of self-tolerance and suppression of autoimmunity. Tregs recognize both self and foreign antigens and operate at sites of inflammation by suppressing the function of effector T cells, thus modulating the intensity and quality of immune responses. Their identification is based on the expression of activation markers and the forkhead transcription factor P3 (Foxp3). The contribution of Tregs to CNS inflammatory diseases has not been previously addressed.

Methods

We investigated the presence and distribution of Tregs in cases of multiple sclerosis (MS), primary CNS angiitis, sarcoidosis, bacterial, viral and parasitic infections, acute infarction and normal brain. Formalin fixed, paraffin embedded sections of surgical and autopsy material were stained with the indirect immunoperoxidase technique using monoclonal anti-Foxp3, CD4 and CD8 antibodies.

Results

CD4+Foxp3+Tregs were present within the perivascular chronic inflammatory infiltrates in primary CNS angiitis and active MS lesions, but not in chronic inactive MS plaques. In sarcoidosis, tuberculosis, viral infections and abscesses, frequent Tregs were identified in perivascular location and scattered in the inflamed tissue. A smaller number were present in parasitic and fungal infections and in leptomeningeal exudates in bacterial meningitis. Tregs were absent in the normal brain and acute ischemic lesions.

Conclusions

The present study shows participation of Tregs in autoimmune and infectious diseases suggesting a complex immune regulatory role in CNS inflammation.

PHENOTYPIC CHARACTERIZATION AND CARDIOVASCULAR OUTCOMES OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Background /objectives

Familial hypercholesterolemia (FH) is a common autosomal dominant disorder caused by loss-of-function mutations in the low-density lipoprotein (LDL) receptor (LDL-R) or apolipoprotein B-100 (apo B) gene, or gain-of-function mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9), resulting in very high blood cholesterol levels and premature cardiovascular disease (CVD).

To identify and phenotypically characterize patients in the Healthy Heart Prevention Clinic with FH to determine characteristics that increase their risk of developing CVD.

Methods

Perform a chart review of the FH patients in the Prevention Clinic and use of the Cardiac Registry to determine which patients in this cohort developed CVD (Group 1).

Results

Preliminary work on 354 patients revealed that 72 had evidence of CVD. The average age of these patients (Group 1) is 69.7 with 58.3% being male. The 282 FH patients not found to have evidence of CVD (Group 2) have an average age of 60.9 with 41.5% being male. Based upon the Dutch Lipid Clinic Network Criteria, 90.3% of Group 1 was defined as having definite FH compared to 54.6% of Group 2. There were some notable differences in CV risk factors between the 2 groups. In the patients of Group 1 62.5% had a family history of premature CVD, 37.5% had history of hypertension, and 59.7% developed tendon xanthomas. In Group 2 51.1% had a family history of premature CVD, 18.8% had a history of hypertension, and 35.1% developed tendon xanthomas. Lastly, there is a large difference in the Lp(a) values of the two groups with Group 1 having an average Lp(a) value of 799.4 mg/L and Group 2 having an average Lp(a) value of 541.1 mg/L.

Conclusions

Diagnosis of definite FH, older age, presence of tendon xanthomas, family history and high Lp(a) levels were much more prevalent in patients who developed CVD. Identifying these risk factors will allow for earlier and more aggressive management to reduce the risk of CVD.

CHARACTERIZATION OF STRESS GRANULES IN THE COXSACKIEVIRUS TYPE B3 INFECTION

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Background /objectives

Coxsackievirus type B3 (CVB3) is a non-enveloped, positive single-stranded enterovirus that infects the heart, pleura, pancreas and liver. CVB3 infection can cause myocarditis, and its sequelae, dilated cardiomyopathy (DCM). In North America, DCM accounts for approximately 20% of heart failure and sudden death in children and young adults. Stress granules (SGs) are dynamic cytosolic aggregates composed of messenger ribonuclearproteins (mRNPs) and target mRNAs and play an important role in mRNA storage and metabolism during stress. SG formation has been implicated in several human diseases including viral infection, inflammation, cancer and multiple neurological diseases. We aim to elucidate the mechanism and significance of SGs in CVB3 infection. Understanding the underlying molecular mechanisms of CVB3 replication and SG formation may aid in developing therapeutic treatments for CVB3-induced diseases.

Hypothesis: CVB3-induced Stress Granule formation plays a protective role in the CVB3 infection

Methods

HeLa cells stably expressing GFP-tagged Ras-GAP SH3 domain binding protein (G3BP1), a protein involved in the assembly of SGs, were used to monitor the formation of SGs during CVB3 infection. To explore the potential mechanism by which CVB3 regulates SG formation, we examined protein expression of eIF4γ(gamma), phospho-eIF2α(alpha), G3BP1, and T-cell restricted internal antigen (TIA-1), another critical protein that participates in SG assembly, in CVB3-infected cells. Finally, RT-PCR, immunoblot and plaque assays were used to investigate the significance of G3BP1 by overexpressing and siRNA knockdown in HeLa cells.

Results

Confocal imaging showed punctate accumulation of GFP-G3BP1 fluorescence in the cytosol at ~3 hours post infection (hpi) and disappearance ~5 hpi, while GFP-G3BP1-Q325E mutants (cleavage resistant mutant) retained SG formation throughout CVB3 infection. By immunoblot, we showed that eIF4α(gamma) was cleaved early after viral infection and eIF2α(alpha) became phosphorylated at ~5 hpi. Protein level of TIA-1 remained unchanged while G3BP1 cleavage was observed ~5 hpi. Finally by RT-PCR, immunoblot and plaque assay, viral replication was significantly reduced in G3BP1 overexpressing cells compared with wild-type HeLa cells. Furthermore, knockdown of G3BP1 resulted in increased viral production.

Conclusions

Our results suggest that translation initiation inhibition is likely the cause of SG formation during the early phase of CVB3 infection, however at the late stage of viral infection, disassembly occurs due to G3BP1 cleavage at amino acid Q325. Our data also suggest a protective role of G3BP1 in SGs during CVB3 infection.

ROLE OF CXCL10 AND ITS RECEPTOR CXCR3 IN COXSACKIEVIRUS B3-INDUCED VIRAL MYOCARDITIS

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Background /objectives

Coxsackievirus B3 (CVB3) is a predominant pathogen in viral myocarditis, an inflammatory disease of the myocardium. In our previous studies using a CVB3-myocarditis mouse model, we found that CXCL10, an interferon (IFN) α-inducible secreted chemokine expressed by immune cells, was dramatically up-regulated during CVB3 infection and the expression level of CXCL10 was always correlated with the severity of myocarditis at the later stage of infection. However, the details about the role of CXCL10 in CVB3-induced viral myocarditis remain obscure. Generally, two mechanisms contribute to pathogenesis of viral infections: 1) direct cell injury due to virus amplification, and 2) damage of the tissue as a result of over-reactive immune response. Our recent data suggest that CXCL10 induces premature cell death and recruitment of NK cells, while persistent over-expression of CXCL10 in the heart initiates exaggerated immune responses which does harm to heart function, hinting that CXCL10 may play dual functional role in determining outcome of CVB3 infection: 1) inhibiting the virus via inducing cell apoptosis and recruitment of NK cells at the early phase of infection, and 2) inducing exaggerated immune responses at late phase of infection, resulting in fetal gene expression and cardiac disruption/remodeling. CXCL10 activate the downstream pathway via interaction with its receptor CXCR3. CXCR3 exists in three alternative splicing forms, A, B and alt. It has been reported that CXCR3-A mediates cell proliferation while CXCR3-B mediates inhibition of cell growth. Hence, we frame our hypothesis that expression of different splicing forms of CXCR3 critically contributes to the dual function of CXCL10 in CVB3 infection: 1) up-regulation of CXCR-B during the early stage of infection activates the pro-apoptotic genes, leading to cell death; 2) up-regulation of CXCR3-A during the late stage of infection results in fetal gene expression and subsequent cell growth.

Methods

1) To detect changes of expression of CXCR-A and CXCR-B in different phases of CVB3 infection, expression level of the two splicing forms during CVB3 infection were measured by RT-PCR in sham- and CVB3-infected HeLa cells. 2) To uncover the relationship between different splicing forms of CXCR3 and CXCL10-induced downstream pathways during CVB3 infection, CXCL10 antagonists were designed and used. Considering the N-terminus of CXCL10 plays a critical role in receptor activation, we designed different CXCL10 antagonists by deleting or changing several amino acids at the N-terminus of CXCL10 and tested if they can attenuate the function of CXCR3 in a CVB3-infected mouse cardiomyocyte HL-1 cell line. Expression of pro-apoptotic gene p53 and its responsive genes p21 and p27, as well as viral capsid proteins VP1 was detected by western blot to serve as the indicator of CXCR-B's function.

Results

Wild-type CXCL10 was shown to up-regulate p21, p27 and p53 and inhibit viral protein VP1 at 16 hours post infection, while this effect could be reversed by CXCL10-Ant-d4, an antagonist with deletion of ten amino acids in the N-terminus of wild-type CXCL10, in a dose dependent manner.

Conclusions

With only quite preliminary data, no critical conclusions can be drawn.

THE PRO-VIRAL ROLE OF AUTOPHAGY IN COXSACKIEVIRUS B3-INDUCED MYOCARDITIS

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Background /objectives

Coxsackievirus B3 (CVB3) is one of the predominant viruses causing myocarditis. Myocarditis and its sequela dilated cardiomyopathy account for ~20% of heart failure and sudden death in children and youth in North America. Autophagy is a cellular process by which organelles/proteins are enwrapped by double membrane vesicles (autophagosomes) and degraded following fusion with lysosomes. Autophagy has been implicated in the modulation of infection and immunity. Some viruses have developed strategies to utilize autophagy to their own ends. We have shown that CVB3 infection up-regulates the formation of autophagosome to facilitate viral replication. In this study we aim to elucidate the role of autophagy in CVB3-induced myocarditis in vivo. We also propose to further understand the underlying mechanisms by which CVB3 exploits the host autophagy machinery for successful replication *in vitro*.

Methods

Autophagy reporter mice with green fluorescence protein (GFP)-labeled microtubule-associated protein 1 light chain 3 (LC3), a marker of autophagy, were used to monitor autophagosome formation. Male GFP-LC3 mice at the age of 4 to 5 weeks were infected intraperitoneally with 104 pfu (plaque-forming unit) of CVB3 for 3 and 9 days. Confocal microscopy and Western blotting were performed to examine autophagosome formation. To further explore autophagic flux, we examined the protein levels of p62. P62 is an adaptor protein mediated selective autophagy pathway by targeting ubiquitinated proteins, organelles and invading pathogens to the autophagy pathway.

Results

Confocal microscopy analysis showed an increase in GFP-LC3 puntates in CVB3-infected GFP-LC3 mouse hearts. In line with this observation, elevated conversion of non-modified LC3-I to lipidated LC3-II was detected by Western blotting. Using antibodies against either C- or N-terminus of p62, we found that CVB3 infection led to marked decreases in the protein level of p62 (~62 kDa), accompanied by the appearance of ~30 kDa fragments. This observation was further confirmed using a flag-tagged p62 construct.

Conclusions

Our results suggest that the autophagy pathway is activated in mouse hearts after CVB3 infection. The autophagy adaptor protein p62 is cleaved during CVB3 infection. Cleavage of p62 may be a viral strategy to establish efficient viral replication in host cells.

MICRORNA-203 ENHANCES COXSACKIEVIRUS B3 REPLICATION THROUGH TARGETING ZINC FINGER PROTEIN-148 AND SUBSEQUENT PROMOTION OF CELL GROWTH

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Background /objectives

Coxsackievirus B3 (CVB3) is the primary causal agent of viral myocarditis. During infection it hijacks host genes to favour its own replication. However, the underlying mechanism is still unclear. **Hypothesis:** Although the viral receptor is an important factor for viral infection other factors such as microRNAs may also play an important role in this biological process.

Objective: To analyse the miRNA profiles in CVB3-infected murine heart and investigate the role of the indentified miRNA in regulating CVB3 replication.

Methods and Results

Microarray analysis using CVB3-infected murine hearts identified miR-203 as one of the most upregulated candidates. We further found that CVB3 induced miR-203 upregulation is through the activation of protein kinase C/transcription factor AP-1 pathway. Ectopic expression of miR-203 downregulated zinc finger protein-148 (ZFP-148) translation and promoted cell growth, which resulted in enhanced CVB3 replication. We further confirmed by luciferase assay that ZFP-148, a transcription factor, is a novel target of miR-203. Silencing of ZFP-148 by siRNA showed similar effects on cell survival and CVB3 replication. Finally, analyses of the signalling cascade downstream of ZFP-148 revealed that miR-203-induced suppression of ZFP-148 differentially regulated the expression of prosurvival and proapoptotic genes of the cell cycle regulators and Bcl-2 family proteins. This altered gene expression promoted cell survival and growth, which provided favourable environment for CVB3 replication and damage of the target cells.

Conclusions

These findings suggest that upregulation of miR-203 during CVB3 infection may be an important regulatory mechanism governing cardiomyocytes growth and thus enhancing CVB3 replication and damage of the heart.

ANALYSIS OF THE EFFECTS OF A REDUCTION IN MYELOID-SPECIFIC STAT6 EXPRESSION ON THE DEVELOPMENT OF ATHEROSCLEROSIS IN LDLR KNOCKOUT MICE

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Background /objectives

Interferon-gamma, signaling through Signal Transducers and Activators of Transcription (STAT) 1, results in classically activated macrophages while interleukin (IL) 4, signaling through STAT6, causes macrophage alternative activation. Although the proatherogenic properties of classically-activated macrophages have been demonstrated in vitro and in vivo, the roles of alternatively-activated macrophages in atherosclerosis remain controversial. We aim to determine the atherogenic potential of murine alternatively-activated macrophages and whether the absence of STAT6 within the myeloid compartment will alter atherosclerosis in low density lipoprotein receptor (LDLR) knockout mice.

Methods

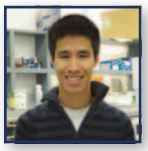
Murine wild type (WT) and STAT6 knockout bone marrow-derived macrophages (BMDMs) were treated with IL4 and then loaded with oxidized low density lipoprotein for cholesterol accumulation assays. Stat6-/-Rag1-/- bone marrow was transplanted into lethally irradiated Ldlr-/- mice to deplete STAT6 in myeloid cells. Mice were fed a high fat diet for 14 weeks then sacrificed for the analysis of atherosclerosis in the aortic root and the descending aorta. The relative abundance of circulating monocyte subsets was assessed by flow cytometry. Mice transplanted with Stat6+/+Rag1-/- bone marrow were used as controls.

Results

There was a statistically significant increase in cholesterol accumulation in IL4-treated WT BMDMs compared to untreated WT BMDMs; this trend was lost in Stat6-/- BMDMs. There was a 16% decrease in aortic root lesion area in mice transplanted with Stat6-/-Rag1-/- bone marrow compared to mice receiving Stat6+/+Rag1-/- bone marrow, although the data did not reach statistical significance. Ldlr-/- mice transplanted with Stat6-/-Rag1-/- bone marrow had significantly decreased pro-inflammatory (Ly6c+CD62L+) and elevated patrolling (Ly6c-CD62L-) monocyte subsets in the blood, bone marrow, and spleen compared to mice transplanted with Stat6+/+Rag1-/- bone marrow.

Conclusions

There was a STAT6-dependent increase in cholesterol accumulation in IL4-treated murine BMDMs compared to untreated cells. The loss of STAT6 in vivo caused a shift in monocyte subsets towards an anti-inflammatory profile.



INTERFERON GAMMA INHIBITS PPAR GAMMA-DEPENDENT UPREGULATION OF CD36

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Background /objectives

Macrophages are heterogeneous in nature and may play different roles in atherosclerosis progression. Macrophage heterogeneity can be modulated by specific cytokine environments. We have previously found that primary monocyte-derived macrophages (MDMs) treated with interferon gamma (IFNg) and tumor necrosis factor alpha (TNFa) in combination and individually accumulate less cholesterol. We sought to determine the individual effects of IFNg and TNFa on the expression of the scavenger receptors CD36 and SR-AI and if IFNg or TNFa were able to prevent the transcription factor peroxisome proliferator-actived receptor gamma (PPARg) from upregulating CD36.

Methods

Primary human MDMs were treated with different combinations of IFNg and TNFa to induce unique phenotypes. MDMs were then treated in the presence or absence of the PPARg-specific agonist rosiglitazone (RSG), and loaded with oxidized low density lipoprotein (oxLDL). After 24 hours of loading, total cellular cholesterol was measured and CD36 protein expression was determined by flow cytometry.

Results

Flow cytometry results showed that either IFNg or TNFa treatment alone reduced CD36 expression (35%, $p<0.05$, and 44% $p=0.006$, respectively) compared to the untreated control. Although no changes in SR-AI mRNA were observed with any cytokine treatment, IFNg and TNFa were associated with a 30% and 68% reduction in SR-AI protein, respectively($p=0.003$, $p=0.03$). RSG treatment resulted in 1.5-fold induction of CD36 in the untreated control ($p<0.05$) and a 1.4-fold induction of CD36 in the TNFa alone condition ($p<0.05$). However, IFNg alone or IFNg in combination with TNFa showed no significant change in CD36 expression ($p=0.14$ and $p=0.17$, respectively).

Conclusions

Treatments containing IFNg impaired the induction of CD36 protein expression by RSG which suggests that IFNg can prevent CD36 expression in a PPARg-dependent manner. The mechanism responsible for the observed changes in SR-AI protein expression is currently under investigation.

THE EFFECT OF PEGYLATION ON INTRACELLULAR SIGNALING OF LYMPHOCYTES

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Background /objectives

The covalent attachment of non-immunogeneic polymers, such as methoxypoly(ethylene glycol) [mPEG] to the surface of cells has been shown to dramatically reduce in vivo antigenic recognition and immunogenicity. Recently, our laboratory demonstrated that PEGylated allogeneic leukocytes induced an immunosuppressive state (increased Tregs and decreased Th17 cells) both in vitro (human and mouse) and in vivo (murine transfusion model). This finding was in stark contrast to the proinflammatory state induced by unmodified allogeneic cells. Thus, PEGylation may be a promising approach in the prevention of allorecognition and the induction of immunotolerance in transplantation. However, our studies to date have not defined the underlying molecular events inducing the tolerogenic response in PEGylated cells. It is our hypothesis that the loss of allorecognition in PEGylated cells results in changes to intracellular signaling cascades giving rise to both altered cytokine secretion and changes in T-cell differentiation.

Methods

Jurkat cells were modified with or without activated 20 kDa mPEG at 0.5 or 2 mM for 60 minutes. Cells were stimulated for 12 minutes with mitogens to trigger proliferation (phytohaemagglutinin [PHA]). Cell lysates were assessed for phosphorylation status using human phospho-kinase array kits. Membranes were analyzed for relative levels of phosphorylated protein using Chemigenius software.

Results

Phosphorylated intermediates important in negative regulation of apoptosis such as Akt and Erk1/2 were significantly decreased in modified cells stimulated with PHA compared to PHA stimulated unmodified cells, as well as unstimulated control cells. This may indicate a lack of inhibition of pro-apoptotic regulators such as Bim and Bad. In addition, the down-regulation of CREB, which is a mediator in the Ras/Raf pathway important for transcription of anti-apoptotic proteins such as Bcl-2 and Bcl-xL, also occurred in modified Jurkat cells.

Conclusions

The initial phosphokinase screen indicates that various intermediates important in cell survival are decreased, suggesting a pro-apoptotic cell fate in modified cells stimulated with PHA. This initial data may indicate important signaling pathways and targets for further investigation.

THE ROLE OF HYPOXIA INDUCED FACTOR 1α AND NOTCH SIGNALING IN ANERGIC T CELL MAINTENANCE DURING HOMEOSTATIC EXPANSION

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Bacground

The process of central tolerance, which weeds out autoreactive CD8+ T cells, is important in preventing autoimmune diseases. The process however, is imperfect, resulting in the release of some of these auto reactive T cells into circulation. It is widely believed that through the tolerizing cytokines secreted by the T regulatory cells and or the continual exposure to the self-antigen without the presence of co-stimulatory signals, these auto-reactive T cells become antigen unresponsive (anergized). This state of anergy was shown to be reversible under certain conditions such as homeostatic expansion (HE), or under the influence of certain cytokines such as IL-7 and or IL-15. In a recent publication by Greenburg et al. these anergized autoreactive T cells were shown to be activated during HE, but revert back to anergy after lymphocyte population was restored independent of cytokines or antigens. Epigenetic regulation was strongly implicated in the study. **We hypothesize that:** the epigenetically programmed CD8+ T cell anergy is driven by Notch signaling and hypoxia induced factor 1α signaling, thus modulating the actions of Notch signaling, and Hif-1a signaling, will affect the establishment/maintenance of their epigenetic memory.

Objectives

To determine whether or not modulating Notch and or Hif-1α signaling could result in the impairment of anergy establishment/maintenance in the autoreactive CD8+ T cells during homeostatic expansion using a rat insulin promoter OVA (RIP-OVA), OT1 TCR transgenic mouse model.

Methods

We will first generate a transgenic mouse strain carrying a specific T cell receptor recognizing a specific self-antigen by crossing OT1 mice with RIP-OVA mice. The anergized OT1 T cells will be harvested from the OT1-RIP-OVA mice using H2Kb-SIINFEKL tetramer and FACS. They will then be treated with HIF-1α activator Desferrioxamine (DFO) before being reintroduced into age-matched, syngenic partially lymphodepleted recipients treated with or without anti-notch ligand antibodies. The autoreactivity of the CD8+ OT1 cells will be monitored in several ways: We will assess the autoreactivity in vivo by monitoring onset of autoimmune diabetes (AD) in the recipient mice after treatment. We will assess the autoreactivity in vitro using ELISA, and chromium 51 assay. The expression levels of the components of the epigenetic machinery and microRNAs will be measured using qPCR, and the epigenetic configuration on the loci of important CD8 T cells effector molecules such as granzyme will be assessed using chromatin immunoprecipitation.

Results

Modulation of Hif-1α and or Notch ligand during homeostatic expansion caused differential expression of the epigenetic machinery. As the result, reactivated OTI CD8+ T cells were no longer anergized after the homeostatic expansion was completed. This defect is manifested in vivo as AD and in vitro as enhanced cytotoxicity and cellularity.

Conclusions

The elucidation of the molecular mechanisms of T cell anergy maintenance allows us to device new strategies in modulating T cell anergy in order to treat diseases such as autoimmunity and cancer.

THE ROLE OF RETINOIC ACID IN MODULATING CD4 T CELL IMMUNE RESPONSES IN THE GUT

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Background /objectives

Dietary vitamin A is essential for the immune system to mount protective responses. Mucosal surfaces, such as that within the gastrointestinal system, are especially vulnerable to dysregulated immunity. Retinoic acid (RA) is a key metabolite of vitamin A enriched at mucosal sites and has potent immunomodulatory effects. RA can promote regulatory T cell (Treg) differentiation by regulating the expression of the transcription factor Foxp3 which is critical for immune homeostasis. RA is also known to upregulate gut-specific homing receptors such as CCR9, important in immune tolerance within the gut. RA cellular levels are largely controlled by Cyp26 enzymes. Since T cells mainly express Cyp26b1 to negatively regulate RA-signaling, we hypothesized that T cells deficient in Cyp26b1 would have enhanced RA-signaling and Treg capacities. Targeting RA-dependent mechanisms within T cells could serve as a therapeutic avenue for treating chronic inflammatory diseases.

Methods

C57BL/6 mice with floxed Cyp26b1 alleles were crossed with Cd4-Cre+ mice to generate mice with conditional deletion of Cyp26b1 specific to T cells. Spleens and lymph nodes were harvested from either wild-type (WT) or conditional knockout (CKO) mice for CD4 T cell isolation and culturing under Treg-polarizing conditions (TGFbeta, IL-2 and αCD3/28 stimulation) with or without all-trans RA added. Cells were analyzed by flow cytometry. To induce chronic colitis, naïve effector T cells (CD4+CD25-CD45RBhi) from donor WT (n=5) or Cyp26b1 CKO (n=6) mice were sorted and injected intraperitoneally into immunodeficient Rag1 mice. Weight loss was monitored and histological processing/staining of diseased colons was carried out at the endpoint. Colons were pathologically scored and relevant tissues were examined for inflammatory markers.

Results

Cyp26b1-/- CD4 T cells had increased Treg differentiation and were more responsive to RA in the presence or absence of exogenous TGFbeta and also had increased CCR9 expression. Interestingly, expansion in the absence of TGFbeta is uncharacteristic of conventional Tregs in vitro. Cyp26b1-/- Tregs generated this way could possibly be more stable, more suppressive, and immunologically more protective. In vivo, Cyp26b1-/- naïve effector T cells caused less disease in Rag1 mice as evidenced by trends of less weight loss and lower histopathological scores of colonic tissue.

Conclusions

These results suggest that Cyp26b1 is an important regulator of RA-signaling in T cells and modulates both regulatory and effector functions. Targeting RA-mediated processes may be a future therapeutic approach for chronic diseases such as Crohn's disease and ulcerative colitis.

ABSTRACT #38

BASIC SCIENCES

GRADUATE STUDENT

POSTER PRESENTATION

ASSESSING NOVEL NKT/TREG CELL THERAPIES TO PREVENT TYPE I DIABETES IN NOD MICE

Jason KS Hung[1], I-Fan Lee[1], Omar Duramad2, Rusung Tan[1]

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Background /objectives

Type I diabetes (T1D) arises from the selective breakdown of immunological tolerance towards pancreatic beta cells and may result from a functional failure of regulatory cells, such as natural killer T (NKT) cells, FOXP3+CD4+ regulatory T (Treg) and dendritic cells (DC). To augment the function of these immunoregulatory cell lineages as a novel T1D therapy, we have developed a novel liposome that is composed of three parts: a liposomal membrane that specifically targets DCs, insulin, which is thought to be the key antigen involved in type I diabetes and a potent activator of Tregs, and the NKT cell agonist alpha-galctosylceramide (aGalCer). **Our overall hypothesis is that simultaneous activation of NKT and Treg cells through DCs using this novel agent will decrease the incidence of T1D or reverse its onset.**

Methods

Female nonobese diabetic (NOD) will be injected with liposomal agents RGI-aGalCer-INS (aGalCer and insulin), RGI-INS (insulin only), RGI-aGalCer (aGalCer only) or PBS (no treatment). Incidence of diabetes will be measured weekly through blood glucose levels, presence of insulin auto-antibodies and islet specific glucose-6-phosphatase catalytic subunit-related protein residue 206-214 (IGRP206-214) and insulin B chain residue 10-18 (B:10-18) specific T cells will be measured using tetramer staining. Mice will be sacrificed at various time points and blood, spleen, lymph nodes and pancreas will be harvested. Frequency and phenotype of NKT, Treg and dendritic cells will be measured with flow cytometry and serum will be collected and cytokine profiles will be measured using a cytokine bead assay. Histology sections will be measured to determine insulitis. Finally, mice will be injected once with the different liposomal agents and sacrificed after 3 days to measure presentation capacity, efficiency and longevity of aGalCer on DCs using an aGalCer:CD1d complex antibody.

Results

Preliminary studies have shown that RGI-aGalCer-INS induces insulin specific Treg proliferation in the pancreas and that spleen cells treated with RGI-aGalCer-INS and then restimulated with insulin peptides have a significant increase in interleukin-2 (IL-2) and interleukin-10 (IL-10) secretion, two cytokines related to the survival, expansion and activation of Tregs.

Conclusions

aGC therapy alone has proven difficult to manage due to its toxicity and insulin peptides have shown the capacity to prevent insulitis but unfortunately not diabetes. Liposomal delivery of αGC has been found to be immune dampening and it's believed that activating both NKT and Tregs simultaneously may have improved application for antigen specific immune suppression and tolerance induction regimens. This research offers a novel treatment that could lead to the creation of new synergistic therapies for T1D and will elucidate the pathways and mechanisms that link DC, NKT and Treg cells together in T1D.

IMPAIRED PROCESSING OF PROIAPP AS A POSSIBLE CAUSE OF RAPID AMYLOID FORMATION IN TYPE 2 DIABETES

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[1]Child and Family Research Institute

Background /objectives

Diabetes is marked by a progressive loss of α-cells that secrete insulin, effectively disrupting blood glucose homeostasis. Islet amyloid polypeptide (IAPP), a hormone processed by the enzymes prohormone convertase 2 (PC2) and prohormone convertase 1/3 (PC1/3) from its proIAPP precursor, accumulates in pancreatic islets as a primary component of amyloid deposits. We have previously shown that proinsulin processing is impaired in the pancreatic beta cell during type 2 diabetes. We hypothesize that because insulin and IAPP maturation are both dependent on enzymes PC2 and PC1/3, processing of proIAPP is also hindered. We aim to determine if accelerated amyloid formation, due to defective proIAPP processing, promotes the development of type 2 diabetes.

Methods

To test our hypothesis, we have developed an ELISA specific to IAPP precursors. We intend to run the ELISA on sera collected from healthy individuals and patients with type 2 diabetes. To model defective IAPP processing in mice, we designed a Pcsk2 conditional knockout construct to allow for beta-cell specific removal of PC2, thus compromising IAPP processing. The plasmid construct was electroporated into embryonic stem (ES) cells derived from a C57Bl/6 mouse. ES clones were screened by PCR and Southern blot, and cells carrying the desired recombination were injected into a blastocyst, which was then transplanted into a C57Bl/6 pseudopregnant mouse. Resulting chimeras were crossed to C57Bl/6 albino mice and black pups were genotyped for the conditional allele. We intend to knockout PC2 expression in beta cells by crossing our conditional line to MIP-CreER mice. These mice will then be crossed to human IAPP expressing mice in order to induce amyloid deposition. To identify early onset of type 2 diabetes in our model, we will measure blood glucose levels and perform glucose tolerance tests. Additionally, we will isolate islets to identify amyloid formation and apoptotic markers.

Results

Precursors are detectable in human islet lysates at a minimum sensitivity of 2.5 islet equivalents. Also, the ELISA generates a signal in sera from rats that overexpress human proIAPP. The total IAPP precursor content in human serum remains undetectable but we are attempting various approaches to overcome this obstacle. The litter that originated from a single injection of our recombined ES cells into the blastocyst developed chimerism, indicating a potential for germline transmission. Crossing of chimeras to C57Bl/6 albino mice yielded black pups carrying the Pcsk2 conditional allele.

Conclusions

Due to limitations in assay sensitivity, we are currently unable to detect IAPP precursors in human serum but are able to identify them in islet lysates and in sera from animals overexpressing human proIAPP. Our conditional mouse model will be invaluable to understanding the role of IAPP intermediates in amyloidogenesis and type 2 diabetes.

LEUKOCYTE TELOMERE LENGTH DURING PREGNANCY AND POSTPARTUM IN HIV-INFECTED WOMEN UPON INITIATION OR INTERRUPTION OF HIV ANTIRETROVIRAL THERAPY

Sara Saberi[1], Beheroze Sattha[1], Deborah Money[2] and Hélène Côté[1]

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Background /objectives

Women of childbearing age constitute ~50% of HIV-infected people worldwide and as many as 3 million HIV-infected women become pregnant every year. While 90% of HIV-infected children acquire the virus through their mother, treatment with highly active anntiretroviral therapy (HAART) during pregnancy and delivery can reduce HIV transmission from ~25% to <2%. Little is known about the possible adverse effects of HAART initiation and interruption on HIV-infected pregnant women. While uncontrolled HIV viremia can cause immune activation and inflammation, nucleoside reverse transcriptase inhibitors (NRTIs) that form the backbone of HAART may inhibit telomerase, the enzyme that elongates and maintains telomere length. Telomerase activity is expressed in hematopoietic stem cells and certain immune cells. Telomere shortening is associated with aging and predicts shorter lifespan. We previously showed that HIV+ children not on HAART have shorter leukocyte telomere length (LTL) that those on HAART.

Hypothesis: In pregnancy, LTL may vary with HAART initiation and interruption.

Objectives: 1. Measure LTL in HIV-infected pregnant women treated with HAART from the second trimester to delivery, and in HIV-negative pregnant controls.

2. Determine whether LTL varies during pregnancy and whether it is influenced by HAART initiation or interruption.

Methods

HIV+ (n=98) and HIV- pregnant (n=57) women were prospectively enrolled in the Pregnancy and CARMA cohorts. Approximately 20% of the HIV+ women conceived on HAART and continued their regimen throughout their pregnancy while the remaining women initiated HAART around the 20th week of gestation. Blood samples were collected around week 18 of gestation (HIV+ women on or off HAART), one month before and at delivery (all HIV+ women on HAART), and 6 weeks post-partum (HIV+ women on or off HAART). S amples were collected at similar intervals from HIV-negative controls. Demographic and clinical data were also collected for all subjects. I will use these samples to measure relative LTL using a qPCR-based assay. I will then compare LTL between periods of pregnancy and between on- and off-HAART periods by paired t-tests. I will also compare LTL between groups by ANCOVA, adjusting for important covariables such as maternal age, HIV history, HAART history, and lifestyle variables such as smoking and drug use.

Results

No studies have examined longitudinal LTL in the context of pregnancy or HAART initiation/ interruption . The result from this research will increase our understanding of the direct effect of HAART initiation and interruption on LTL. It will also help tease apart the effects of uncontrolled HIV vs. HAART on blood telomere maintenance.

ULTRASTRUCTURAL DAMAGE TO THE LIVERS OF HEPATITIS C VIRUS AND HIV CO-INFECTED INDIVIDUALS

Hayley Spencer Hiebert[1,2], David Schaeffer[2], Rachel Wade[2], Fanny Chu[1], Helene Cote[2] and David Walker[1,2]

[1]Providence Heart + Lung Institute at St. Paul's Hospital; [2]Department of Pathology and Laboratory Medicine, UBC; [3]Department of Pathology, Mount Sinai Hospital, Toronto

Background /objectives

In the developed world, end-stage liver disease is the leading cause of death for patients infected with Human Immunodeficiency Virus (HIV). Among HIV-infected individuals, the Hepatitis C Virus (HCV) coinfection rate in Vancouver is estimated to be in excess of 90%. HCV chronicity often results in accelerated liver fibrosis, cirrhosis and/or liver failure. Management of HIV infection by HAART (Highly-Active Anti-Retroviral Therapy) involves nucleoside-analog reverse transcriptase inhibitors (NRTIs), some of which exert toxic effects on the mitochondria and decrease mitochondrial DNA (mtDNA). The anti-viral combination therapy for HCV includes another NRTI, ribavirin, used in conjunction with pegylated interferon. To investigate the direct effects of anti-HCV combination therapy in individuals on and off-HAART, we conduct morphometric and qualitative ultrastructural analyses via transmission electron microscope (TEM) images to characterize liver biopsy specimens obtained before and after anti-HCV combination therapy. The mtDNA content is also investigated.

Methods

Two ultrasound-guided liver biopsies were obtained from coinfected patients before and after HCV combination therapy, and scored by a single, blinded pathologist for fibrosis and inflammation. Biopsy tissue was also used for DNA analysis and TEM imaging via a FEI Tecnai 12 TEM at 80kV. mtDNA content analysed by quantitative PCR was expressed as a ratio of mtDNA to nuclear DNA. TEM processing of the biopsy involved fixation in 2.5% glutaraldehyde, sodium cacodylate buffered solution. Specimens were post-fixed with osmium and potassium ferrocyanide, dehydrated, infiltrated, and embedded in epoxy resin (Epon). Image-Pro software was employed for morphometry of the digital images, with a blinded single examiner.

Results

Changes in the hepatocytes volume following treatment were evident, but did not correlate to treatment outcome, use of HAART, or to HCV genotype. The volume fraction of lipid decreased in each case, irrespective of HCV genotype or HAART usage. The analysis of mitochondrial hypertrophy is ongoing at present. Changes in the ratio of mtDNA to nuclear DNA were not significant.

Conclusions

We present preliminary data suggesting that the livers of coinfected individuals may experience a decrease in hepatosteatosis following anti-HCV combination therapy, regardless of HAART status or treatment outcome.

ORAL EPITHELIAL CELL TELOMERE LENGTHS (TL) ARE CORRELATED WITH PERIPHERAL BLOOD LEUKOCYTE TL IN HIV+ AND HIV-EXPOSED UNINFECTED (HEU) CHILDREN, AND ARE NOT AFFECTED BY HIV VIREMIA

DeAnna Zanet[1], Hugo Soudeyns[3], Ariane Alimenti[4], Valérie Lamarre[3], Normand Lapointe[3], Jason Brophy[6,7], Lindy Samson[6,7], Deborah M Money[1,2,4], Anona Thorne[5]; John C Forbes[4], Hélène CF Côté[1,2] and the CIHR Emerging Team in HIV therapy and aging (CARMA)

[1]UBC; [2]WHRI; [3]Centre de Recherche du CHU Sainte-Justine, Montreal [4]B.C. Women's Hospital; [5]CIHR Canadian HIV Trials Network; [6]Children's Hospital of Eastern Ontario; [7]University of Ottawa

Background /objectives

Telomeres cap and protect the ends of chromosomes, and shorten with each somatic cell division, acting as a marker of aging. Telomerase, homologous to HIV reverse transcriptase, is expressed in germ stem and stem cells, and maintains telomere length (TL). Nucleoside reverse transcriptase inhibitors (NRTIs) are given to HIV+ pregnant women for their own health and to prevent mother to child transmission of the virus. HIV-exposed uninfected (HEU) infants also receive NRTI prophylaxis for 6 weeks after birth. NRTI inhibition of telomerase and HIV-induced inflammation and/or oxidative stress can adversely affect TL maintenance, and possibly influence tissue aging. We previously showed that although peripheral blood leukocyte (PBL) TL was similar between HEU and HIV+ children, the latter demonstrated shorter TL if they had a detectable HIV plasma viral load. This study examined whether similar TL dynamics are observed in buccal epithelial cells.

Methods

HIV+ and HEU children were prospectively enrolled in the CARMA cohort. Mouth swabs and PB were collected from each child and their relative TL was measured by qPCR. Univariate linear regressions examined relationships of potential predictor variables with TL. Three multivariate models were created: one for all subjects, and two others that included HIV+ and HEU subjects only.

Results

Oral epithelial cell (OEC) TL data was obtained for 90 HIV+ (median [IQR] age (years) 13.1 [9.7-15.6]) and 231 HEU (1.6 [0.5-3.3]) children. All HEU children were exposed to NRTI in utero and/or neonatally. OEC TL was ~70% longer ($p<0.0001$, paired t-test) than and correlated with PBL TL (Pearson's $r2=0.29$, $p<0.0001$). In a multivariate analysis for all subjects that included age, gender, HIV status, parental ages and ethnicity, older age was associated with shorter OEC TL ($p<0.001$). In addition, compared to Caucasians, children of Aboriginal ethnicity had shorter OEC TL ($p=0.05$) while Blacks had longer OEC TL ($p=0.03$). Among HEU, OEC TL was not associated with duration of in utero ART. Among HIV+ children, although immune parameters were predictive univariately, only older age ($p=0.003$) predicted shorter OEC TL multivariately.

Conclusions

Our preliminary results did not detect differences in OEC TL between HIV+ and HEU children but this reassuring observation is limited by the age imbalance between the groups. OEC TL was unrelated to the length of antenatal NRTI exposure in HEU. Furthermore, unlike our previous observation with PBL TL, OEC TL was not related to HIV plasma viral load in HIV+ children. This suggests that immune activation rather than systemic inflammation may explain the association between short PBL TL and HIV viremia.

A NOVEL POLYMERIC ANTIDOTE FOR CLINICALLY USED HEPARIN ANTICOAGULANTS

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[1]Department of Pathology and Lab Medicine, UBC; [2]Department of Chemistry, UBC

Background /objectives

Heparin based parenteral anticoagulants such as unfractionated heparins (UFH) and low molecular weight heparins (LMWHs) are widely prescribed for the treatment and prophylaxis of thromboembolic complications. Unfractionated heparin is also used extensively for anticoagulation therapy during major invasive surgeries such as cardiopulmonary bypass. Potential risk of bleeding and heparin induced thrombocytopenia associated with UFH administration led to the development of novel parenteral anticoagulants such as fondaparinux with superior safety profile. Nonetheless, haemorrhage is the major adverse effect associated with all available anticoagulants and hence administration of reversal agents is inevitable. Haemorrhage could be life threatening in geriatric, pediatric populations and patients with coagulation disorders and renal insufficiency. The only clinically approved antidote available is protamine sulphate which neutralizes UFH. However, life threatening cardiovascular adverse reactions of protamine and ineffectiveness in completely neutralizing novel anticoagulants restricts its use as an antidote. Hence, there is an imperative need to develop biocompatible and efficient universal antidote which could neutralize all available anticoagulants. Here, we report development of hyperbranched polyglycerol based biocompatible cationic polymers capable of reversing in vitro anticoagulation effect of UFH and LMWHs.

Methods

Cationic polymeric neutralizing agents with hyperbranched polyglycerol (HPG) core and polyethylene glycol chains (PEG) were synthesized by protocol developed in our laboratory. Synthesized polymeric antidotes were fully characterized by gel permeation chromatography (GPC), nuclear magnetic resonance (NMR) and conductometric titrations. Activated partial thromboplastin time (aPTT) assay was performed in human platelet poor plasma and anticoagulated human platelet poor plasma respectively to assess biocompatibility and neutralization capability of polymeric antidotes. Biocompatibility was also assessed by performing erythrocyte aggregation, hemolysis and complement activation assays. Thromboelastography (TEG) measurements were performed to investigate the heparin neutralization capacity of polymeric antidotes in human whole blood.

Results

Developed cationic polymeric antidotes completely neutralized the anticoagulation effects of UFH and LMWHs in vitro over a broad concentration ranging from 0.025mg/mL to 1mg/mL. On the other hand protamine failed to completely reverse anticoagulation effects of LMWHs and also showed anticoagulation effect above 0.1mg/mL. Polymeric antidotes also exhibited excellent hemocompatibility compared to protamine as they did not induce hemolysis, erythrocyte aggregation or activate the complement system.

Conclusions

We have developed a novel, biocompatible and efficient polymeric reversal agent capable of neutralizing in vitro anticoagulation effects of UFH and LMWHs. Development of antidotes for synthetic pentasaccharides and ultra-low molecular weight heparins (ULMWHs) such as fondaparinux and semuloparin are in progress.

A HIGH THROUGHPUT SCREEN TO IDENTIFY NOVEL ENHANCERS OF INDUCED PLURIPOTENT STEM CELL FORMATION

Damian Yap[1,2], Jazmine Edwards[1], Adrian Wan[1], Peter Tong[3], Anthony Arnoldo[4], Jason Moffat[4], Corey Nislow[4], Andras Nagy[3] & Sam Aparicio[1,2]

[1]Department of Pathology and Laboratory Medicine, UBC; [2]Department of Molecular Oncology, BCCRC; [3]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto; [4]Donnelly Centre & Molecular Genetics UofT

Background /objectives

Recent work showed that differentiation was not only reversible but that it was possible to reprogramme mouse embryonic fibroblasts (MEFs) to become induced pluripotent stem cells (iPS) using four defined factors, Oct4, Sox2, c-Myc and Klf4. The presence of oncogenic c-Myc explained why mice generated from such iPS cells developed tumuors, thus limiting potential clinical applications. In this study, we sought to identify replacers of c-Myc which when ectopically expressed were able to reprogramme MEFs expressing only Oct4, Sox2 and Klf4 (3-factor MEFs) within 12 days.

Methods

We generated a robust inducible system in 3-factor MEFs which make iPS cells only when supplemented with ectopically introduced c-Myc. We performed the screen by substituting c-Myc with Lenti-viral pools expressing open reading frames (ORFs, in 42 pools) and selecting for iPS colonies. Using next generation sequencing, we identified the ORFs required to replace c-Myc in the generation of iPS clones from 3-factor MEFs. The individual ORFs were then re-introduced to 3-factor MEFs to generate iPS cells and the resulting iPS colonies were validated using established assays and biomarkers of pluripotency.

Results

We isolated 57 individual iPS clones in which some ORF(s) have effectively replaced c-Myc in the generation of iPS from 3-factor MEFs within the given time frame.

Conclusions

The identity of these ORFs implicate processes and pathways in the reprogramming enhanced by c-Myc. The replacement of c-Myc in the efficient generation of iPS may move such technology another step in the direction of *in vivo* applications.

THE ROLE OF GRB2 IN INTEGRIN-INDUCED PROTEIN TYROSINE PHOSPHATASE ALPHA (PTPA) TYR-789 PHOSPHORYLATION

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Background /objectives

Integrins are heterodimeric transmembrane receptors which upon binding to the extracellular matrix (ECM) transduce extracellular signals to the inside of a cell to regulate cell adhesion, migration and survival. The integrin signaling cascade is characterized by a series of protein tyrosine phosphorylation events that are tightly regulated by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Defective integrin signaling is often associated with tumor cell behavior such as metastasis. Thus, a detailed understanding of the molecular mechanisms involved in integrin signaling will provide insights for development of anti-cancer therapeutics. Protein tyrosine phosphatase alpha (PTPa) is a receptor PTP that plays two roles in integrin signaling. Its catalytic activity contributes to its role as an activator of the Src family tyrosine kinases, and its phosphorylation at the C-terminal tail Tyr-789 modulates actin stress fiber assembly, focal adhesion formation, and cell migration.

Recent work in our lab has shown that phosphoTyr789 of PTPa is required for PTPa translocation to focal adhesions (FAs), however the molecular mechanism of PTPa FA recruitment remains unclear. PTPa phosphoTyr789 is known to interact with the SH2 domain of Grb2 and this association occurs in an integrin-dependent manner. In this study, our objective is to elucidate the role of PTPa-associated Grb2 and we hypothesize that Grb2 recruits PTPa to FAs via its binding to PTPa-phosphoTyr789.

Methods

We used Grb2 siRNA to knockdown Grb2 expression in wild type mouse embryonic fibroblasts (MEFs) to study the function of Grb2 in integrin signaling. We performed focal adhesion enrichment assays, co-immunoprecipitations, and immunofluorescent microscopy to investigate the molecular defects in integrin signaling in the absence of Grb2. We also constructed Grb2 siRNA resistant plasmids to re-express wild type and mutant forms of Grb2 in Grb2 knockdown cells to determine which domain of Grb2 is important for its effect on PTPa.

Results

We found that silencing Grb2 expression (>90% efficiency) significantly reduces PTPa-Tyr789 phosphorylation and cell migration. Surprisingly, integrin-stimulated PTPa translocation to FAs is unaffected in Grb2 knockdown cells, even though the phosphorylation of PTPa at Tyr789 is abolished. We also established that Grb2 does play a role in maintaining PTPa in FAs. Reintroduction of wild-type and mutant forms of Grb2 into Grb2 knockdown cells revealed differential requirements for Grb2 domains in regulating integrin-stimulated PTPa Tyr789 phosphorylation.

Conclusions

Grb2 is not required for the recruitment and maintenance of PTPa in FA but its SH2 and C-terminal SH3 domains are required for PTPa-Tyr789 phosphorylation. Further investigations to elucidate the molecular mechanisms of integrin-dependent PTPa recruitment to FAs and Grb2-regulated PTPa-Tyr789 phosphorylation are in progress.

SELECTIVE INHIBITION OF DNA-PK BY A HYPOXIA ACTIVATED PRODRUG

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Background /objectives

Hypoxia is a common feature of solid tumours imparting overall resistance to radiotherapy (RT). Hypoxic cells are approximately three times more resistant to RT compared with normoxic cells. Hypoxic cells can be sensitized to ionizing radiation (IR) by inhibition of DNA-dependent protein kinase (DNA-PK), a holoenzyme that is a central component of non-homologous end joining (NHEJ), one of the mechanisms responsible for repair of DNA double-strand breaks (DSB's). The objective of this study was to develop a hypoxia activated inhibitor of DNA repair.

Methods

1-(2-hydroxyl-4-morpholin-4-yl-phenyl)ethanone (IC86621) was used as a prototype drug DNA-PK inhibitor and was conjugated to a hypoxia-activated trigger moiety which releases the DNA-PK inhibitor selectively under hypoxic conditions. Experiments were carried out to evaluate the effect of the prodrug on radiosensitization of cells at differing levels of oxygenation. Cell suspensions of human non-small cell lung carcinoma (NSCLC) H460 cells and mouse liver microsomes were used to investigate cofactor requirements, metabolic kinetics and oxygen dependence on prodrug bioreduction. The resazurin reduction assay was used to assess the prodrugs impact on radiosenstivity of HeLa cells following IR under hypoxic (0% O_2) and aerobic conditions (air). Immunohistochemical staining of γH2AX was performed to examine the presence of persistent DSB's following prodrug and inhibitor treatment. Clonogenic assays with H460 cells were performed to assess radiosensitization and cytotoxicity. Preclinical pharmacokinetics were studied in C3H/HeN mice following single intraperitoneal or oral gavage administrations.

Results

Stirred cell suspensions and microsomal incubations confirmed that IC86621 is released from the prodrug selectively under hypoxic conditions and efficient cleavage occurs at <0.2% O_2. This process was determined to be an NADPH dependent process. The presence of γH2AX foci 35 h post treatment with prodrug and inhibitor confirm the presence of persistent DSB's following treatment. Clonogenic assays determined that the prodrug sensitizes cells with equivalent efficiency to that of the inhibitor selectively under hypoxic conditions. Pharmacokinetic analysis shows that prodrug concentration in the blood peaks 1 h following administration with a t½ of ~3 h for oral gavage.

Conclusions

Our results indicate that targeting DNA repair through selective inhibition of DNA-PK is a promising approach to sensitizing radioresistant hypoxic cells.

SOX15 IS A NOVEL TUMOR SUPPRESSOR GENE IN PANCREATIC CANCER

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Background /objectives

Pancreatic ductal adenocarcinoma (PDAC) ranks among the top five deadliest cancers in the world. To rationally identify new therapeutic targets, the genes and pathway driving pancreatic tumourigenesis must be elucidated and understood.

Objective: To identify novel tumour suppressor genes that are disrupted in PDAC and contribute to pancreatic tumourigenesis.

Methods

DNA and RNA from 20 pancreatic cancer cell lines and the non-malignant, human pancreatic ductal epithelial (HPDE) cell line were used to generate DNA copy number, DNA methylation, and gene expression profiles. For each PDAC line we looked for candidate tumour suppressor genes that were under-expressed and inactivated by "two-hits" at the DNA level (i.e. copy number loss and hyper-methylation) within the same cell line. We then looked across the 20 cell lines to identify those recurrently disrupted in at least 3 (15%) PDAC lines. To further refine our candidate gene list, we filtered for genes that were under-expressed in at least 20% of pancreatic tumours using publicly available expression data. We subjected genes satisfying these criteria to Ingenuity Pathway Analysis. The contribution of SOX15 to pancreatic tumour biology was assessed using in vitro and in vivo models.

Results

We identified 61 candidate tumour suppressors undergoing two-hits in at least 3 PDAC cell lines and under-expression in at least 20% of pancreatic tumours. Of these, SERPINB13 and SOX15 were the most frequently disrupted by two-hits (≥45% of cell lines). Pathway analysis for these candidates revealed they were significantly enriched for involvement in the Wnt pathway. Other SOX family members are known to suppress Wnt-stimulated transcription, therefore, we focused on SOX15. Re-expression of SOX15 in a PDAC cell line with undetectable mRNA and protein expression resulted in reduced viability in vitro and smaller tumour size and volume in vivo compared to the empty-vector control line, demonstrating its tumour suppressive capability.

Conclusions

We identified SOX15 as a novel tumour suppressor gene that is frequently inactivated in pancreatic cancer and thought to function at the transcriptional level in the Wnt pathway; thus, SOX15 status may be important to consider when using therapeutics that target Wnt signaling.

ARSENIC-RELATED GENOMIC AND EPIGENOMIC ALTERATIONS IN LUNG SQUAMOUS CELL CARCINOMAS

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Background /objectives

Arsenic is a well-established skin, bladder, and lung carcinogen. It is estimated that over 100 million people worldwide are exposed to toxic levels of arsenic, mainly through drinking water. Lung cancer is the main cause of deaths due to arsenic exposure, with this metalloid acting as the major etiological agent in cancers that occur in never smokers. Relative to other subtypes of lung cancer, lung squamous cell carcinomas (SqCCs) occur at higher rates following arsenic exposure.

The mechanisms by which arsenic causes cancer is still under investigation, and it has been proposed that both genetic and epigenetic processes may be involved.

In this study, a rare panel of lung tumours from a population with chronic arsenic exposure, including SqCC tumours from patients with no smoking history, was analyzed to identify arsenic-associated copy-number alterations (CNAs) and DNA methylation changes.

Methods

A total of fifty-two cases of lung SqCC were analyzed by whole-genome tiling-set array comparative genomic hybridisation. Twenty-two specimens were collected from arsenic-exposed patients from Northern Chile (10 corresponding to never smokers and 12 to smokers). A panel of thirty cases was obtained for comparison from North American smokers without arsenic exposure. In addition, 22 blood samples from healthy individuals from Northern Chile were examined to identify naturally occurring germline DNA copy-number variations (CNVs) that could be excluded from analysis. DNA methylation analysis was performed using Illumina's Infinium Human Methylation 450K array.

Results

We identified several CNAs and DNA methylation changes associated with arsenic exposure. These alterations were not attributable to either CNVs or smoking status. The most recurrent events represented DNA losses at chromosome bands 1q21.1, 7p22.3, 9q12, and 19q13.31. The only arsenic-associated DNA gain occurred at 19q13.33, which contains genes previously recognized as oncogenes.

Conclusions

Our study has provided insights into the molecular mechanisms of arsenic-induced lung neoplasia. The distinct and recurrent arsenic-associated genetic and epigenetic alterations suggest that this group of tumours may represent a separate disease subclass.

POSTER PRESENTATION

MULTI-OMICS INTEGRATED TUMOR-GENOM RANKING ANALYSIS

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Background /objectives

The purpose of this project is to develop a system to identify key "driver" genes and signaling pathway which they disrupt, in order to better understand the molecular mechanisms responsible for driving the lung cancer phenotype.

Methods

We developed a novel scoring algorithm called Multi-omics Integration Tumour-genome Ranking Algorithm (MITRA) and applied it to previously generated DNA copy number, DNA methylation and gene expression profiles for matched lung tumor and non-malignant lung tissue samples from the same individuals to identify the most probable driver genes in the individual cases. This algorithm gives higher score/weight to genes which are disrupted by multiple mechanisms at the DNA level and concurrently show concordant expression changes at the RNA level. We then evaluated the efficacy of our algorithm by assessing its ability to identify cancer pathways (using Ingenuity Pathway Analysis). We compared pathway results generated using gene expression profiles only (traditional method) against those generated using MITRA derived genes.

Results

The genes which received higher scores from the MITRA algorithm included not only genes known to be components of important cancer pathways, but also novel genes, which escaped detection by conventional gene expression profiling analyses. The MITRA strategy, therefore, is capable of identifying new gene candidates that may be key to cancer development, substantiating the role of MITRA in cancer gene discover and hypothesis generation.

Conclusions

Using the MITRA algorithm, candidate driver genes can be identified in the context of biological pathways which may be important to cancer development. Successful application of MITRA to lung cancer samples illustrate the utility of this algorithm for gene discovery using multi-omics data in cancer reaserch.

INTEGRATIVE GENOMIC ANALYSIS OF MIRNAS IN LUNG ADENOCARCINOMA

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Background /objectives

Lung cancer represents an enormous health burden, representing the most common cause of cancer death worldwide, with a five-year survival of less than 15%. micro RNAs (miRNAs) have emerged as major players in lung cancer oncogenesis, displaying both oncogenic and tumor suppressive functions. DNA copy number (CN) amplification of oncogenes is a major molecular mechanism driving cancer, and like protein coding genes, CN alterations can influence miRNA expression levels. We hypothesize that DNA copy number gain modulates the expression of miRNAs important to cancer cell growth, and that integrative analysis can identify these cancer driving miRNAs.

Methods

Global gene dosage profiles for 46 lung adenocarcinoma and paired adjacent non-malignant tissues were generated by array comparative genomic hybridization. miRNA sequencing analysis was performed on this same panel of tumours and matched non-malignant tissues using Illumina GAXII small RNA sequencing technologies. CN and expression were correlated for each miRNA (Spearman's correlation >0.3, $p<0.05$) and expression of each significant miRNA was compared between tumours with and without CN gain (U-test $p<0.05$). To select for miRNAs that most likely play a role in cancer we compared the expression between tumour and matched normal and selected for miRNAs with a fold change of at least 2 in tumours as compared to normal tissues.

Results

We identified a panel of miRNAs in lung adenocarcinoma whose expression deregulation was associated with CN alteration. These miRNAs were found to be gained in >15% of tumours and underwent at least a 2 fold greater increase in expression in tumours compared to matched normal tissues. This study identified several miRNAs known to play a role in cancer including miR-141, known to be highly upregulated in lung and ovarian cancer, and miR-301a, upregulated in invasive early cervical cancer. In addition we identified several novel miRNAs that have not yet been implicated in cancer.

Conclusions

Here we identify a panel of miRNAs that are selectively gained at the genomic level to alter expression in lung adenocarcinoma. Several of these miRNAs have been previously shown to have oncogenic properties in many cancer types including lung cancer and may be potentially useful as therapeutic targets. In addition we identify several miRNAs that have not been previously reported in lung cancer. Future characterization of these miRNAs may lead to increased knowledge of adenocarcinoma oncogenesis.

EPI-002 ACCELERATES LIGAND DISSOCIATION FROM ANDROGEN RECEPTOR BY DISRUPTING N-TERMINUS TO C-TERMINUS INTERACTION

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Background /objectives

Constitutively active splice variants of androgen receptor (AR) lacking the ligand-binding domain (LBD) are linked to the development and progression of castration recurrent prostate cancer (CRPC). Recent studies suggest a constitutively active splice variant, ARv567es, is capable of interacting with full-length AR (ARfl), stabilizing and enhancing its ligand dependent activities. EPI-001, an AR antagonist targeting the N-terminus domain (NTD) prevents N-terminus to C-terminus (N/C) interaction of AR, which is essential for AR antiparallel dimer formation. The ligand-dependent N/C interactions slow the dissociation of ligand from the LBD. Here we examine the effect of EPI-002, a stereoisomer of EPI-001, on ARv567es complexed with full-length AR and test the hypothesis that EPI-002 will cause ligand to dissociate more quickly because it blocks N/C interaction.

The aim of this study is twofold. First we will examine the effects of AR^{v567es} on the dissociation rate from the full-length receptor. Then we will examine the effect of EPI-002 on the ligand dissociation rate of AR^{fl} with and without the presence of AR^{v567es}.

Methods

Ligand dissociation rates were examined in live cells using a radiolabelled synthetic androgen, 3H-R1881. Ligand dissociation assay was used to investigate the effect of ARv567es on the dissociation rate of 3H-R1881 from wild-type full-length AR (wt AR^{fl}) transfected in Cos-1 cells. We also examined the effect of EPI-002 on the dissociation rate of 3H-R1881 from 1) endogenous AR^{fl} in LNCaP cells with a point mutation (codon 868, Thr to Ala), 2) wt AR^{fl} in Cos-1 cells and 3) mixture of wt AR^{fl} and ARv567es at 50:50 ratios in Cos-1 cells. Expressed AR proteins were detected by Western blot analysis.

Results

AR^{v567es} had no effect on the dissociation of ligand from LBD. EPI-002 increased the ligand dissociation rate from endogenous ARfl in LNCaP and wt ARfl by ~13% and 30%, respectively. 30% inhibitory effect was observed on mixture of ARfl and AR^{v567es} at 50:50 ratios. Levels of AR protein achieved by transfection were within normal physiological levels when compared to levels of endogenous AR expression in LNCaP cells.

Conclusions

Consistent and optimal levels of expression of full-length to variant AR can be achieved by transient transfection. EPI-002 quickens the dissociation of androgen from mutant full-length AR, wild-type full-length AR and the mixture of wild-type full-length and variant AR by disrupting N/C interaction. Together these data begin to reveal the potential of EPI-002 for the treatment of CRPC in which most patients have numerous tumors, each with varying levels of expression of full-length and variant AR.

EPI COMPOUNDS INHIBIT TRANSCRIPTIONAL ACTIVITY OF ANDROGEN RECEPTOR SPLICE VARIANTS

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Background /objectives

Of the 25,500 Canadian men that are diagnosed yearly with prostate cancer, 20-40% will progress to castration-resistant prostate cancer (CRPC) and succumb within 2 years of disease onset. Growth and progression of CRPC is dependent on the transcriptional activity of the androgen receptor (AR). Recurrence to this aggressive and lethal form of the disease is caused by resistance to current androgen deprivation therapies through adaptation events such as alternative splicing of AR, which forms truncated proteins that lack the full ligand-binding domain (LBD). AR V567es and V7 are the most prominent and clinically relevant splice variants that are correlated with poor prognosis in patients with bone metastasis. Since current androgen deprivation therapies target the LBD, a novel amino-terminal domain (NTD) small molecule inhibitor that blocks transcriptional activity of the splice variants would significantly improve the management of CRPC. Small molecule EPI-001 is a mixture of 4 stereoisomers that bind to the AR NTD. EPI-002 is the most active stereoisomer of EPI-001. Our aim is to characterize the interaction of AR variants, V567es and V7, with full-length AR in prostate cancer cell lines and evaluate EPI compounds as novel AR inhibitors. By inhibiting NTD transcriptional activity of these constitutively active AR variants, EPI compounds will be assessed as potential therapeutic agents for CRPC.

Methods

AR negative monkey kidney cell line, COS-1, were transiently transfected. Western blot analysis was employed to validate expression of splice variants V567es and V7 using an antibody to the AR NTD epitope residues 1-20. To measure transcriptional activity of the full-length AR and splice variant V567es AR driven-reporter genes, prostate-specific antigen (PSA)-luciferase and probasin(PB)-luciferase, were co-transfected in androgen sensitive human prostate LNCaP cells and treated with the antiandrogen bicalutamide that binds to the AR LBD, or EPI compounds that bind the AR NTD in the presence and absence of synthetic androgen R1881.

Results

Expression of splice variants V567es and V7 was detected at a lower molecular weight (approximately 80 and 70 kDa, respectively) than native full-length AR at 110 kDa. We demonstrated that EPI-001 inhibited both ligand-dependent full-length AR and constitutive AR V567es transcriptional activity in LNCaP cells. Both EPI-001 and EPI-002 effectively inhibited transcriptional activity of V567es in a dose-dependent manner in transiently transfected AR COS-1 cells.

Conclusions

EPI-001 and its stereoisomer EPI-002 are currently the only known drug therapies to inhibit AR constitutively active splice variants lacking AR LBD. EPI compounds may benefit those patients failing current therapies that target the AR LBD. Future studies will include investigation of the interaction between the AR V7 and full-length AR, and characterization of EPI compounds on V7 activity.

CLINICAL PERFORMANCE OF THE PROEX C MINICHROMOSOME MAINTENANCE PROTEIN AND TOPISOMERASE 2A (MCM-TOP 2A) BIOMARKER TEST IN DETECTING CERVICAL INTRAEPITHELIAL NEOPLASIA

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Background /objectives

The clinical usefulness of the ProEx C test (Becton Dickinson) for the detection of high-grade cervical intraepithelial neoplasia or worse (CIN 2+) was determined in comparison with the PreTect HPV-Proofer E6/E7 mRNA assay (Proofer, Norchip) and Hybrid Capture 2 HPV test (HC2; Qiagen) in a multicentre study carried out in Canada.

Methods

The study population comprised of women with a history of abnormal cytology referred to colposcopy. Cervical specimens were collected in PreservCyt (ThinPrep; Hologic) and SurePath (BD) media. PreservCyt was utilized for cytology using the ThinPrep method, and processed for Proofer and HC2 testing. SurePath was processed for ProEx C. Histology-confirmed CIN 2+ served as the disease endpoint and gold standard.

Results

The study analysis was based on 1360 women (mean age 30.7 years), of who 380 (27.9%) had a histological diagnosis of CIN 2+. The sensitivity of ProEx C was 73.9% (95% confidence interval [CI]: 72.8, 75.1) whereas it was 76.1% for Proofer (95% CI: 74.9, 77.2) and 95.5% (95% CI: 95.0, 96.1) for HC2. When stratified by cytological grades, the sensitivity of ProEx C for detecting CIN 2+ ranged from 69.2% in atypical squamous cells of undetermined significance (ASCUS) to 67.6% in low-grade squamous intraepithelial lesions (LSIL) and 89.1% in high-grade intraepithelial lesions (HSIL). The corresponding figures were 72.5%, 74.1%, and 83.2% for Proofer, and 86.9%, 95.6%, and 99.0% for HC2. ProEx C showed an overall specificity of 71.6% (95% CI: 70.4, 72.9) versus 75.9% (95% CI: 74.8, 77.1) for Proofer and 38.2% (95% CI: 36.8, 39.5) for HC2.

Conclusions

The performance of ProEx C was found to be similar to that of Proofer but more specific than HC2 for detecting CIN 2+ and hence could significantly reduce colposcopy referral of both ASCUS and LSIL cytology, albeit with a lower sensitivity compared to HC2.

SCREENING OF THYMIC EPITHELIAL TUMORS FOR COMMON MUTATIONS USING NEXT GENERATION SEQUENCING

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Background /objectives

Molecular pathogenesis of thymomas and thymic carcinomas is poorly understood. No commonly occurring mutations have been identified so far, although a small percentage of thymic carcinomas have activating mutations in c-Kit and EGFR. The objective of our study is to screen ten thymomas and two thymic carcinomas for common mutations in oncogenes and tumour suppressor genes using next generation sequencing technology. The Ion Torrent PGM platform permits massively parallel sequencing and "deep interrogation" of the cancer genome. Current work flow around 5 days makes this an emerging technology a potentially useful tool in clinical molecular diagnostics.

Methods

Two thymomas from each of the five WHO classes (A, AB, B1, B2, and B3) and two thymic carcinomas were selected from a large series of patients diagnosed with thymoma or thymic carcinoma in British Columbia between 1994 and 2009, which were all fully characterized clinically and pathologically. H&E slides and formalin-fixed paraffin-embedded (FFPE) blocks were obtained from the original institutions. Cores were obtained from areas of pure tumour type and DNA was extracted. 10ng of DNA from each specimen is subjected to multiplex PCR for 739 potential mutations in 46 oncogenes and tumour suppressor genes (AmpliSeq Cancer Panel). Libraries of the amplicon products are generated using emulsion PCR and are sequenced using the 100Mb chip. Sequencing data is compared with the hg19 reference genome and variants are identified.

Results & Conclusions

The results of the analysis of the sequencing data are not available at the time of this abstract's submission. Results and conclusions will be presented at the Pathology Day 2012. This project highlights the potentials of next generation sequencing technology as a useful adjunct in clinical pathology practice. The ability to perform deep interrogation of the cancer genome from FFPE material makes this especially powerful.

PRENATAL RAPID ANEUPLOIDY DETECTION: WHAT IS THE BRITISH COLUMBIA FUTURE LANDSCAPE?

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Background /objectives

Prenatal cytogenetic diagnosis is offered to BC women with: 1) advanced maternal age; 2) a positive maternal screen for chromosomal aneuploidy; or 3) an abnormal fetal ultrasound as all of these women have an increased risk to have conceptions with trisomy 21, 18 and other chromosomal abnormalities.

Karyotyping is currently performed but is a labour intensive and expensive technique. Rapid aneuploidy detection (RAD) of the common prenatal aneuploidies (trisomy 21, 18, 13 and sex chromosome numerical abnormalities) can be performed by fluorescence in situ hybridization (FISH) or by quantitative fluorescent polymerase chain reaction (QF-PCR). Both tests have the same turn-around-time (1-3) days, but RAD is reported to be less expensive. QF-PCR has been clinically performed in other countries and Canadian provinces, not including BC, for several years. Our objective was to validate the QF-PCR technique to introduce more efficient ways to perform prenatal genetic diagnosis in BC.

Methods

Two commercial kits were tested on amniotic fluid DNA received from 3 other laboratories and on left over amniotic fluid samples available in the laboratory.

Results

A total of 370 samples were available for validation. Preliminary results show no false positive and no false negative results. The final results will be presented.

Conclusions

QF-RAD is a rapid and cost efficient technique for the detection of common prenatal aneuploidies. This technique is done routinely in some Canadian provinces and countries. The scenarios for prenatal genetic diagnosis differ: in some countries, eligible pregnant women can choose QF-PCR or karyotyping. In others, both techniques are offered to eligible women, while other centres have replaced karyotyping with QF-PCR for low-risk pregnancies (pregnancies with positive prenatal genetic screening and advanced maternal age). The scenarios for prenatal genetic diagnosis are quickly increasing, with the possibility of performing genetic screening tests with high positive predictive value.

POPULATION-BASED ANALYSIS OF CHROMOSOMAL ABNORMALITIES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background /objectives

Chromosomal abnormalities in chronic lymphocytic leukemia (CLL) can be detected by fluorescence in situ hybridization (FISH) in 80% of patients and are important prognostic indicators. Deletion 17p and deletion 11q predict a poor prognosis, trisomy 12 and a normal FISH result confer an intermediate risk and deletion 13q indicates a good prognosis. Furthermore, IGH translocations [t(IGH)], are a recurrent cytogenetic finding in CLL; however, their prognostic significance is unclear. CLL FISH testing is performed at one of 3 sites in BC: Vancouver General Hospital, BC Cancer Agency and Royal Columbian Hospital and all CLL patients receive uniform evaluation and therapy. Our goal is to create a single, unified provincial CLL database that houses comprehensive clinical and laboratory data on all BC CLL patients. With this data we will: 1) determine the prevalence of recurrent chromosomal abnormalities detected by FISH in the BC CLL population both within the entire cohort and stratified by lab test site; 2) assess the impact of t(IGH) on the other chromosomal abnormalities in CLL and 3) review the clinical significance of other parameters including age, sex, percentage abnormal nuclei and clonal evolution in our population.

Methods

Results of the first FISH test from 815 patients referred for testing at VGH, BCCA or RCH between March 2004 to the end of 2011 were reviewed. Patients were diagnosed by finding of lymphocytosis (>5x10^9/L) on bloodwork, morphology, and/or clinical features indicative of CLL (lymphadenopathy, organomegaly, and/or B symptoms), and confirmed by flow cytometry. Clinical, laboratory and demographic data collected by review of hospital, clinical and individual physician's records included: date of diagnosis, CBC and Rai stage at diagnosis, date and type of first treatment, first flow cytometry results, date of death or last follow-up, age and sex. Survival analysis for overall survival (OS) and treatment-free survival (TFS) was performed using SPSS software.

Results

Chromosomal abnormalities were detected in 79% (642/815) of CLL patients with frequencies as follows: deletion 13q (54%), trisomy 12 (19%), deletion 11q (10%), deletion 17p (9%) and t(IGH) (8%). 47 patients were found to have t(IGH). Determination of the impact of t(IGH) on patients with concomitant 13q deletions showed that t(IGH) and deletion 13q had worse treatment-free survival vs. patients with deletion 13q alone, p = 0.004. Results of the other clinical parameters will be presented.

Conclusions

The frequency of recurrent chromosomal abnormalities in CLL as detected by FISH in our cohort is similar to the reported frequency in the literature; however, the percentage of CLL patients with a t(IGH) is significantly higher in the VGH population. t(IGH) appears to negatively impact the good prognosis of deletion 13q further stratifying this risk group. Results of the other clinical parameters tested are likely to provide additional information about CLL and may identify other novel risk factors. In summary, the development of a unified provincial CLL database is an important step to gaining further insight into the patho- and clinicobiology and genetics of CLL and provide a foundation to generate novel research questions.

DEVELOPMENT OF A BIOBANK RESOURCE CENTRE (BRC) BY THE OFFICE OF BIOBANK EDUCATION AND RESEARCH (OBER) AND THE CANADIAN TUMOUR REPOSITORY NETWORK (CTRNET)

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Background /objectives

The Office of Biobank Education and Research (OBER) is a provincial initiative of the Department of Pathology and Laboratory Medicine, University of British Columbia. OBER's goals are: 1) to promote certification of B.C. tumour and non-tumour biobanks in order to enhance quality through standardization and foster public confidence in biobanks; 2) to facilitate adoption of best practice-based standards through education; and 3) to provide active support for new and established biobanks. To address our third goal, OBER created a Biobank Resource Centre (BRC) in collaboration with the Canadian Tumour Repository Network.

Methods & Results

The BRC consists of: 1) live biobank support and 2) a needs/issue assessment strategy and online tool, 3) a fit-for-purpose document library, "tool-kit", and services intended to support all phases of biobanking. Documents include process maps, plans, procedures, equipment and performance catalogue, and facilities design plans. Tools include a biobank user fee costing tool; and Biospecimen Reporting for Improved Study Quality (BRISQ) tool. Services include a biobank pathology annotation and analysis service, and biobank business plan development.

Conclusions

OBER has been established as a centre to communicate common standards and policies amongst biobanks and between biobanks and the public through education, training and support in the form of the BRC. For more information on OBER and the BRC please visit www.ober.pathology.ubc.ca and www.biobanking.ca.

BIOBANK CERTIFICATION: DEVELOPMENT OF A PROGRAM BY THE CANADIAN TUMOUR REPOSITORY NETWORK

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Background /objectives

One goal of CTRNet is to improve the capacity/quality of cancer biospecimens and data through standardization of biobanking processes. CTRNet achieves this by creating national standards to promote the ability of cancer researchers to utilize tumour biobanks. Two areas of focus in the past year have been creation of a Biobank Certification Program linked to an Education Program.

Methods

CTRNet developed a Biobank Certification Program comprising of two linked phases, Registration and Certification - and a supporting Biobank Education Program, both targeting the full spectrum of tumour biobanks. The program design was formulated over a period of 2 years after international landscape assessment, national consultation with a range of stakeholders, advice from leaders from the ethics and research communities, and input from working groups drawn from leaders and staff of leading Canadian biobanks interested in such a process to help ensure public confidence in biobanking and quality of biospecimens for research.

Results

The Registration phase of the CTRNet Biobank Certification Program was launched on November 30th, 2011. Currently, 20 biobanks and 64 individuals across Canada have completed CTRNet Registration and the introductory education module. Researchers can enroll in the program by completing the registration form and the introductory `Basics of Biobanking' education module. The Certification phase of the program is currently in the pilot stage and will be launched in late 2012. For the biobank this will involve completing an extended registration form to enable classification of the type of biobank, completing up to nine assigned educational modules as relevant to the biobank, familiarization with the CTRNet Required Operational Practices, and compiling the relevant SOPs to support these practices. CTRNet's certification will attest that the biobank has undertaken to comply with national standards.

Conclusions

The benefits of deployment of a certification program are widely accepted across biobank, research and ethics communities to foster biobank standardization, ethics review and public confidence, and increased quality in translational cancer research.

SEGMENTAL ANALYSIS OF GAMMA- HYDROXYBUTYRATE IN HAIR USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Background /objectives

Gamma-hydroxybutyrate (GHB) is a drug in the sedative-hypnotic class and its depressive and amnesic effects allow it to be used for drug facilitated sexual assaults (DFSA). Currently, there lacks a practical and standardized way for the detection of single GHB exposure in alleged DFSA since both urine and blood screens have an extremely small detection window. In addition, useful cut-off points for the detection of GHB in blood, urine, and hair are not well-defined because GHB is also an endogenous compound found normally in our body with high variability. For these reasons, the segmental analysis of GHB in hair was proposed to be potentially helpful for the detection of GHB exposure in DFSA. The aim of this study was to investigate the suitability of the liquid chromatography tandem mass spectrometry (LC/MS/MS) method developed for the segmental analysis of GHB in hair.

Methods

A total of 85 specimens were collected from volunteers not claiming any exposure to GHB. The specimens were washed with methanol, hot water, and dichloromethane prior to clipping. 10 sequential segments (3mm and 10mg each) were clipped from each specimen to conduct the segmental analysis of GHB. Standards, quality controls, and blanks were prepared from the blank hair sample. The hair segments were first solubilised in 1N NaOH at 85 degrees Celsius. After cooling, GHB was extracted using liquid-liquid extraction with ethyl acetate. The quantification of extracted GHB was carried out using LC/MS/MS. Our LC/MS/MS (4000QTrap, ABSciex) method was conducted using atmospheric pressure chemical ionization (APCI) in the negative mode, multiple reaction monitoring and deuterated standard, GHB-D6. The calibration curves obtained from the standards were used to quantify the amount of GHB found in each segment of hair. The limit of quantification (LOQ) of this method is 0.1ng/mg. The sequential hair segment profiles were analyzed using Change-Point Analyzer and based on the results, a set of detection criteria was proposed.

Results

For all the samples that gave results greater than the LOQ, the intraindividual variation range is 7% to 44 %, in relative standard deviation, which is consistent with previously published data. The true negative rate of the detection method devised from the Change-Point Analyzer results was found to be at least 97.6% as it correctly identified 83 out of 85 known negative specimens. 2 out of 85 specimens can be possibly mistaken as positives but it was noted that overall circumstantial evidence must be considered when making the final decision.

Conclusions

Our data indicate that the LC/MS/MS method developed is suitable for the segmental analysis of GHB in hair. It was also noted that the Change-Point Analyzer might help in establishing a standardized approach for the interpretation of analytical results in DFSA cases concerning a single exposure of GHB.

VALIDATION OF THE RANDOX TOTAL BILE ACIDS ASSAY ON THE VITROS 5600

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Background /objectives

Intrahepatic cholestasis of pregnancy (ICP) is a serious liver condition affecting 0.2% of pregnant women presenting in the second and third trimesters. It may lead to increased risk of fetal distress, premature birth, and stillbirth. Total bile acids (TBA) is the most sensitive diagnostic marker. Mild and severe ICP are defined as TBA from 11-39 µmol/L and ≥40 µmol/L, respectively.

Objective: To set up and evaluate a commercial enzyme cycling assay for TBA (5th generation) on the vitros 5600 instrument (Ortho-Clinical Diagnostics, Rochester NY), using the user defined assay option.

Methods

Standard method evaluation was conducted. Sixteen samples collected for investigation of ICP were tested using the Randox reagent on the Vitros and compared to results on a reference assay (Trinity Biotech, Bray, Ireland). Samples from 120 fasting pregnant women (gestational ages 22-33 weeks), and 34 random prenatal screening samples (15-20 weeks gestation) were tested to verify the reference range.

Results

The Randox/Vitros method was negatively biased relative to the reference method (y=0.58x+5.0, r2 = 0.97). Calibrators showed similar bias. Precision at levels of 0.65, 23 and 42 µmol/L was 20.5%, 2.2% and 2.6% respectively. Linearity was documented in mixed samples and up to tenfold dilution. TBA was between 0.31-7.82 µmol/L in 151 women, with two random and one fasting measurement above this range.

Conclusions

Different TBA assays give significantly different results, calling into question the decision limits suggested for severity of ICP. The Randox reference range of 2-10 umol/L for fasting TBA is appropriate for pregnancy.

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