Pathology Day

May 27, 2011

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ABSTRACT BOOK / POSTERS & ORAL PRESENTATIONS

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a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA
pathology laboratory medicine

KEYNOTE SPEAKER

Dr. Dario Vignali, PhD | Member, St. Jude Faculty, Vice Chair, Immunology

PRESENTATION TITLE

"Immune Regulation, T-regs and Inhibitory Receptors"

Biography

Dario A Vignali received his Ph.D. from the London School of Hygiene and Tropical Medicine, University of London, England, and his postdoctoral training from the German Cancer Research Centre in Heidelberg, Germany and Harvard University in Cambridge, Massachusetts, USA. He is currently a Vice Chair and Member of the Immunology Department at St. Jude Children's Research Hospital in Memphis, Tennessee, USA. His research focuses on various aspects of T cell biology including TCR:CD3 signalling and cell biology, the development and function of regulatory T cells, and type 1 diabetes.

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Organization

Department of Immunology
St. Jude Children's Research Hospital, Memphis, TN

Education

PhD - London School of Hygiene & Tropical Medicine, University of London

Research Interests

Regulatory T cells and Inhibitory Molecules [identification of novel regulatory T cell (T_R) molecules; mechanism of T_R function, especially by IL-35 and LAG-3; biology and mechanism of action of IL-35 and LAG-3; modulation of the response to tumors, intestinal parasites and asthma by T_R and inhibitory molecules].

T cell receptor (TCR):CD3 complex [molecular initiation and control of signal transduction; role of CD3 in T cell development and function; role of ubiquitinylation in modulating TCR signaling and transport].

Type 1 diabetes [importance of TCR specificity and affinity in the diabetogenic or regulatory potential of T cells; mechanisms that regulate T cell islet entry and diabetogenicity; prevention of diabetes by making insulin-producing b cells that are resistant to immune attack].

JAMES HOGG LECTURE

Dr. James C. Hogg, Professor Emeritus

PRESENTATION TITLE

"Centrilobular emphysematous destruction in Chronic Obstructive Pulmonary Disease (COPD)"

Biography

Dr. Hogg is a celebrated pioneer in the field of lung disease research. A UBC professor emeritus of Pathology and Laboratory Medicine, Dr. Hogg's career spans more than 35 years.

He is known worldwide for breakthrough discoveries in dealing with asthma and chronic obstructive pulmonary disease.

At St. Paul's Hospital, he founded the Pulmonary Research Laboratory. It was renamed in 2003 as the James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research in his honour. At present, the iCAPTURE Centre has more than 250 researchers working on solving the problems of heart, lung and blood vessel diseases.

Dr. Hogg continues to inspire students and the medical community. He is a talented leader and a dedicated mentor. He gives of himself and readily shares his vast experience.

Dr. Hogg was recently inducted into the Canadian Medical Hall of Fame, one of only 76 Laureates to have this honour. Other accolades include the prestigious American Society for Investigative Pathology Chugai Award. Dr. Hogg is also a fellow of the Royal Society of Canada and an Officer of the Order of Canada.

Organization

Department of Pathology & Laboratory Medicine
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Education

MD The University of Manitoba 1962
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DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

UBC Pathology Day 2011

THANKS to everybody who made this event possible and a great experience!

Acknowledgment

Pathology Day is a team effort and we would like to extend special thanks to all those who contributed to the 2011 edition. These include Jennifer Xenakis, Maureen Barfoot, Barbara Sherman, Farrah Rooney, Tony Lin and Sarah Anning for their help organizing this event. Debbie Bertanjoli took care of our website tools and prepared the abstract book. Thanks to Jenny Tai and Greg Doheny for technical support, as well as the volunteers who assist with various tasks and help throughout the day. Finally, we wish to express our gratitude to the many people who contributed their time and expertise reviewing the abstract, moderating the oral sessions and judging the oral and poster presentations. This year, these include Ken Berean, Amanda Bradley, Cedric Carter, Zaph Colby, David Granville, Wam Lan, Cheng Han Lee, James Lim, Haydn Pritchard, Morris Pudek, Jacquie Quandt, Marianne Sadar, Wes Shreiber, Brian Skinnider, Patrick Tang, Cristina Tognon, Peter van den Elzen, Rebecca Walters, and Jennifer Xenakis.

We hope you enjoy Pathology Day 2011.

Hélène Côté and Michael Nimmo
Co-Chairs, Pathology Day 2011

The Pathology Day is an annual event that features poster presentations from graduate students, residents, and other trainees. The day concludes with the annual departmental dinner.

pathology
laboratory medicine

Welcome

A Message from the Head

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 of 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 local and international research excellence and continuing in the tradition of having world leaders in biomedical research speak at Pathology Day. Dr. Jim Hogg (University of British Columbia) will give the James Hogg Lecture: “Centrilobular emphysematous destruction in Chronic Obstructive Pulmonary Disease (COPD)”, while Dr. Dario Vignali (Department of Immunology, St. Jude Children’s Research Hospital, Memphis, TN) is our Keynote Speaker. I extend my thanks and gratitude to Drs. Helene Cote and Mike Nimmo, Co-Chairs of Pathology day, as well as all the other individuals for their efforts in organizing this year’s event.

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

Conference Outline

08:00 Breakfast

08:15 Opening remarks

08:30 James Hogg lecture by Dr. James Hogg (Junior AB Ballroom)
Centrilobular emphysematous destruction in Chronic Obstructive Pulmonary Disease (COPD)

1 Resident Oral Session, Junior AB Ballroom

1 **09:45** Chris Conklin
Ovarian small cell carcinoma hypercalcemic type: exploring the genetic basis for this aggressive neoplasm

2 **10:00** Anna Lee
Loss of BAF250a (ARID1A) is frequent in high-grade endometrial carcinomas

3 **10:15** Salwa Elmalti
Diagnostic usefulness of necrotic bone marrow aspirates and biopsies: immunohistochemical, flow cytometric, cytogenetic and FISH studies

4 **10:30** Hans Frykman
TRAF 6 mediated resistance to panitumumab or cetuximab in stage IV colon cancer patients with KRAS wild type tumor

15 min break

5 **11:00** Majid Mz Zolein
Reality check: a review of 167 cases of adrenal vein sampling in British Columbia

6 **11:15** Tyler Smith
Using evidence and expert consensus to clarify the clinical confusion surrounding heritable thrombophilia testing

7 **11:30** Titus Wong
Community and hospital rates of group B streptococcus resistance to penicillin, erythromycin and clindamycin in Vancouver, Canada

8 **11:45** Vincent Fung
The impact of individual clinical risk factors in predicting 10-year fracture risk in Canadian women of various age groups using fracture risk assessment tool (FRAX)

12:00 **LUNCH**

13:00-15:00 **POSTER SESSION**

9 **15:00** Tony Ng
The AMPK stress response pathway mediates anoikis resistance through inhibition of mTOR and suppression of protein synthesis

10 **15:15** Dmitry Turbin
A Computer Program for Nuclear Chromatin Analysis

11 **15:30** Vivien Wei
Clonogenic analysis of genes that are synthetically lethal with Breast Cancer 2 gene (BRCA2)

12 **15:45** Tawny Hung
Increased mitotic rate and Ki67 expression in ulcerated melanocytic nevi

Graduate Students and Post-Doctoral Fellows Oral Session, Junior D Ballroom 2

13 **09:45** Paul Hiebert
Granzyme B contributes to extracellular matrix remodeling and skin aging in apolipoprotein E knockout mice

14 **10:00** Alon Hendel
Granzyme B disrupts endothelial cell adhesion to fibronectin

15 **10:15** Clara Westwell-Roper
Blockade of interleukin-1 signalling improves human islet amyloid polypeptide-induced pancreatic islet graft dysfunction

16 **10:30** Loraine Bischoff
Recruitment of T regulatory cells to islet grafts prevents recurrent autoimmune diabetes

15 min break

17 **11:00** Melissa McConechy
Identification of subtype-specific somatic mutations of PPP2R1A in endometrial and ovarian carcinomas

18 **11:15** Jennifer Choo
An immunohistochemical survey of biomarkers for basal-like breast cancer against a gene expression profile gold standard

19 **11:15** Amal El Naggar
Insights into the role of the Y-box binding protein-1 (YB-1) in sarcomas

20 **11:45** Kevin Yang
Small molecules to inhibit prostate cancer proliferation by targeting androgen receptor

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Herpesviruses enhance fibrinogen clot lysis

23 **15:30** Hayley Spencer
Investigation of ultrastructural changes in the liver of hepatitis C virus and HIV co-infected patients undergoing HCV therapy

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Ethnicity, adiposity and cardiovascular risk factor are associated with vitamin D status

Pathology Day Keynote Lecture, Junior D Ballroom 3

16:30 Dr. Dario Vignali
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Reception, Dinner and Awards at Law Court 4

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Oral Presentations

Department of Pathology and Laboratory Medicine Pathology Day is an annual event held in late May. Presentations are in oral and poster format and major prizes are awarded at an evening reception. The selection of award winners are made by the judges who review and evaluate the presentations.

There are a number of Awards presented at this event:

- Best three poster presentations
- Best three graduate oral presentations
- Best three resident oral presentations

May , 2011

Chris M.J. Conklin[1], Sohrab P. Shah[2], Martin Kobel[2], Mark Sun[2], Nataliya Melnyk[2], Blaise Clarke[3], Carrie Symmes[4], Doug Pelanda[4], Karey Shumansky[2], Jamie Rosner[2], Alireza Moussavi[2], Leah Prentice[2], David Huntsman[2]

[1]Department of Pathology, University of British Columbia; [2]Centre for Translational and Applied Genomics of BC Cancer Agency, Vancouver, BC; [3]Department of Pathology, University of Toronto; [4]Children's Nationwide Hospital, Columbus, Ohio

Chris Conklin

OVARIAN SMALL CELL CARCINOMA HYPERCALCEMIC TYPE: EXPLORING THE GENETIC BASIS FOR THIS AGGRESSIVE NEOPLASM

Background/objectives

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

Methods

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

Results

Chromosomal analysis did not reveal any significant deletions, duplications, amplifications, or structural abnormalities. Transcriptome analysis is currently ongoing. However, preliminary results reveal point mutations in genes associated with Wnt, p53 and MAPK pathways.

Conclusions

Using next generation sequencing, this is the first complete view of the OSCCHT genome. Based on preliminary results and chromosomal analysis, OSCCHT appears to be a point mutation driven tumor. Results are ongoing.

Anna Lee

Kimberly C Wiegand[1,2], Anna F Lee[2,3], Osama M Al-Agha[3,4], Christine Chow[4], Steve E Kalloger[2], David W Scott[1], Christian Steidl[1], Sam M Wiseman[2,5], Randy D Gascoyne[1], Blake Gilks[2,3,4], David G Huntsman[1,2,4]

[1]BC Cancer Agency; [2]University of British Columbia; [3]Vancouver General Hospital; [4]Genetic Pathology Evaluation Center; [5]St. Paul's Hospital

ABSTRACT #2

LOSS OF BAF250a (ARID1A) IS FREQUENT IN HIGH-GRADE ENDOMETRIAL CARCINOMAS

Background/objectives

Mutation of the ARID1A gene and loss of the corresponding protein BAF250a has recently been described as a frequent event in clear cell and endometrioid carcinomas of the ovary. We sought to determine whether BAF250a loss is common in other malignancies.

Methods

Immunohistochemistry (IHC) for BAF250a was performed on tissue microarrays (TMAs) in more than 3000 cancers, including carcinomas of breast, lung, thyroid, endometrium, kidney, stomach, oral cavity, cervix, pancreas, colon, and rectum, as well as endometrial stromal sarcomas, gastrointestinal stromal tumours, sex cord-stromal tumours and four major types of lymphoma (diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, mantle cell lymphoma, and follicular lymphoma). Whole tissue sections of cases of atypical hyperplasia and atypical endometriosis were also assessed for BAF250a expression by immunohistochemistry. Comparison of BAF250a expression in the tumor types was done using Fisher's exact test (2-tailed).

Results

BAF250a loss was observed in 29% (29/101) of Grade 1 or 2, and 39% (44/113) of Grade 3 endometrioid carcinomas of the endometrium, 18% (17/95) of uterine serous carcinomas, and 26% (6/23) of uterine clear cell carcinomas. In contrast, BAF250a loss in the other examined tumors was less frequent. No loss of BAF250a was seen in the 9 cases of atypical endometrial hyperplasia examined. However, one of 10 cases of atypical endometriosis showed BAF250a loss; this case was the only one that recurred as endometrioid carcinoma.

Conclusions

Loss of BAF250a expression is a particular feature of carcinomas arising from endometrial glandular epithelium, and may occur as an early event in carcinogenesis.

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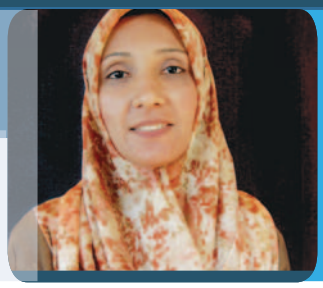

Salwa Elmalti

DIAGNOSTIC USEFULNESS OF NECROTIC BONE MARROW ASPIRATES AND BIOPSIES:IMMUNOHISTOCHEMICAL, FLOW CYTOMETRIC, CYTOGENETIC AND FISH STUDIES

Background/objectives

The diagnostic utility of necrotic bone marrow tissue (NBMT), both aspirate and biopsy, is not established except in scattered case reports. The objective of the current project was to determine the usefulness of immunohistochemistry (IHC), flow cytometry (FCM), conventional karyotyping (CK) and fluorescent in situ hybridization (FISH) in NBMT.

Methods

All the patients in Vancouver General Hospital records coded as "bone marrow necrosis" were included in this study. The CBC, blood films and notes from gross examination of the bone marrow tissue were reviewed for indicators of necrosis such as leukoerythroblastic state (LES), pyknotic leukocytes (PL), presence of marrow particles and necrotic appearance. The bone marrow biopsies were scored for the extent of necrosis, fibrosis and osteosclerosis. A tissue microarray (TMA) was prepared from the biopsy specimens, IHC using 35 markers was performed and scored independently by 2 investigators. FCM data was reviewed to assess the background, scatter pattern and expression of the antigens; the latter was compared with IHC results. FISH was performed on TMA using centromere probe for chromosome 8 and myc probes and scored by a technologist. The bone marrow regeneration time was assessed as the time for the absolute neutrophil count (ANC) to reach 1x10A9/L.

Results

Thirty cases with NBMT from 27 patients were reviewed. The age (22-88yrs, median 55 yrs) and sex (15 males and 12 females) were not different from the parent group. The blood film (LES in 4/30 cases, PL in 0/30 cases) or gross observation of the bone marrow aspirates (pauciparticulate in most cases and grossly necrotic in 2/30) were generally not predictive of necrosis. The diagnosis in 27 patients was acute myeloid (n=12) or lymphoid (n=2) leukemia, lymphoma (10 cases), and 1 each of chronic myeloid leukemia, rhabdomyosarcoma and metastatic carcinoma. 27/30 patients had >50% of the biopsy showing necrotic intertrabecular tissue, while two had necrotic bony trabeculae as well. Sixteen cases had fibrosis, and of them three also had osteosclerosis. 32/35 antibodies worked both in necrotic and neighboring viable tissue; Ki67, CD3 and Vimentin worked only in neighboring viable tissue. FCM showed high background and loosening of clusters of neoplastic cells, but was diagnostic in all five cases. A diagnosis was possible based on IHC and flow cytometry all cases. CK failed to provide dividing cells in 7/11 cases. A signal for centromeric probe for chromosome 8 was detected in 13/27 (48%) of cases; the negative cases were those fixed in B5. The median time to ANC recovery (1x109/L) was 25 days (range 15-36 days).

Conclusions

IHC and FCM yield diagnostic findings in most cases of NBMT. CK works in 1/3 of cases. FISH on the biopsy works better than FISH on aspirate or CK; however, FISH does not work on B5 fixed tissue.

ABSTRACT #3

Hans Frykman[1], Sandy Sung[2], Sharlene Gill[3], and Aly Karsan[2,3]

[1]UBC, [2]BCCRC and [3]BCCA

Hans Frykman

ABSTRACT #4

TRAF 6 MEDIATED RESISTANCE TO PANITUMUMAB OR CETUXIMAB IN STAGE IV COLON CANCER PATIENTS WITH KRAS WILD TYPE TUMOR

Background/objectives

In British Columbia, patients with stage IV colorectal adenocarcinoma are offered either Panitumumab or Cetuximab as a third line treatment if their tumor has tested negative for KRAS mutation (ie. be of wild type). However, despite this targeted therapy, a significant percentage of these patients progress on treatment. Recent discovery suggest that, TRAF6 over amplification on chromosome 11q13 and it's subsequent over expression result in NF-kB activation, malignant cell transformation and tumor formation. Furthermore, TRAF6 has been shown to be critical for RAS mediated malignant transformation and tumor formation.

We postulate that some of the patients with KRASwt who progress on Panitumumab or Cetuximab have a tumor that over amplifies/overexpress TRAF6 and that this is the reason their tumor is resistant to said therapy.

Methods

We thus identified 5 non-responders and 9 responders from patient medical records at the BC cancer agency. Tumor DNA from each tumor in the cohort was isolated from paraffin blocks and analyzed using a customized Affymetrix microarray.

Majid Zolein[1], and Daniel T. Holmes[1]

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Majid Mz Zolein

REALITY CHECK: A REVIEW OF 167 CASES OF ADRENAL VEIN SAMPLING IN BRITISH COLUMBIA

Background/objectives

To retrospectively review the success of adrenal venous sampling (AVS) procedures performed at 6 hospitals across British Columbia (BC) in the past 7 years.

Methods

Aldosterone analysis for all AVS cases in BC are performed at a single tertiary care centre, allowing identification of all subjects. Data included: hospital performing procedure, ordering physician, interventional radiologist, demographics, venous aldosterone and cortisol results - both pre and post ACTH stimulation where applicable.

Results

Patients were 61% M and 38% F with a median age of 53.3y [IQR: 45.3-58.2]. Biochemically-proven success rates of catheterization of the right adrenal vein by site ranged from 27.7% [CI: 10.7-53.6, n=18] to 93% [CI: 81.3-97.6, n=54]. Sites employing ACTH stimulation demonstrated markedly better provable catheterization success. In 10% of cases, although aldosterone results clearly demonstrated catheterization success, standard cortisol-based biochemical criteria for catheterization failed to prove the same. ACTH rescued an otherwise uninterpretable procedure in 22 (13%) of cases, half of which showed clear lateralization. However, stimulation significantly decreased the median lateralization index in the 25 cases showing lateralization (11.8 [9.3,21.8] to 7.1 [3.7,11.0], $p<0.01$, Wilcoxon) and led to a conflicting interpretation in 7 cases.

Conclusions

AVS should be performed by a single dedicated radiologist at each site. Catheterization criteria based on cortisol may fail in a minority of cases. Biochemical proof of catheterization can be improved with ACTH but this may cause apparent decreases in lateralization and a change in interpretation in some cases.

Tyler Smith[1], David Pi[2], Monika Hudoba[2], Agnes Lee[3]

[1]UBC Hematopathology, [2]Hematopathology Dept., Vancouver General Hospital;
[3]Vancouver Coastal Health Thrombosis Program

Tyler Smith

USING EVIDENCE AND EXPERT CONSENSUS TO CLARIFY THE CLINICAL CONFUSION SURROUNDING HERITABLE THROMBOPHILIA TESTING

Background/objectives

Standard heritable thrombophilia testing (HTT) assesses patients for the presence of factor V Leiden, prothrombin gene mutation, and deficiencies of antithrombin and proteins C and S. Unfortunately, it is often unclear how the results of HTT should influence patient management. This has led to significant variability in physician practice and often inappropriate ordering of HTT.

Methods

Our primary goal was to adapt evidence-based HTT recommendations published by Baglin et al in April 2010 into provincial clinical guidelines. To determine the consensus and relevance of these recommendations in BC, we conducted an anonymized online survey of specialists with expertise in thrombosis to quantify their agreement or disagreement each recommendation. Responses were scored using a 5-point Likert scale ranging from -2 (strongly disagree) to +2 (strongly agree). We also introduced a decision-making tool to guide HTT ordering for inpatients at Vancouver General Hospital.

Results

A participation rate of 65% (20 of 31) was received. Of the 30 recommendations, 15 (50%) received strong support, 10 (33%) received moderate support, and 5 (17%) received weak support, as defined by mean scores of 1.0-2.0, 0.50-0.99, and 0-0.49, respectively. Our VGH inpatient HTT tool reduced inpatient testing by approximately 75%.

Conclusions

These results indicate that inpatient HTT is frequently inappropriately requested and that there is sufficient agreement amongst local thrombosis experts with published guidelines to warrant their adaptation into provincial HTT guidelines. Successful completion of this project will lead to reduced inappropriate HTT utilization and serve as a model of knowledge transfer aiding the development of future guidelines targeting other areas of clinical ambiguity.

T. Wong[1], S. Dhaliwal[2], G. Al-Rawahi[1,2], C. Pienaar[1,3], M. Kelly[1,3], L. Martinez[3], P. Tilley And R. Reyes*[1,3]

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Titus Wong

COMMUNITY AND HOSPITAL RATES OF GROUP B STREPTOCOCCUS RESISTANCE TO PENICILLIN, ERYTHROMYCIN AND CLINDAMYCIN IN VANCOUVER, CANADA

Background/objectives

Intrapartum chemoprophylaxis for Group B Streptococcus (GBS) colonization decreases GBS neonatal sepsis mortality. American data have demonstrated resistance rates as high as 32% for erythromycin and 20% for clindamycin. The purpose of this study was to determine the susceptibility patterns of penicillin, clindamycin, and erythromycin for GBS in Vancouver, Canada.

Methods

Consecutive GBS-screening samples were collected from LifeLabs Medical Services, a large community laboratory, and BC Children's and Women's Health Center (BCCWH) from April through November, 2010. Standard microbiology methods, including Prolex kits were used for GBS identification. Penicillin Etest MIC, disk diffusion for erythromycin and clindamycin and D-test were performed and interpreted according to CLSI M100-S20. The susceptibility patterns from both centers were compared.

Results

All BCCWH (n=118) isolates were penicillin susceptible, with a mean MIC (µg/mL) of 0.060, median MIC of 0.064, and MIC90 of 0.064. At LifeLabs (n=163), one isolate had a penicillin MIC of 0.125; mean MIC was 0.064, median MIC was 0.064, and MIC90 was 0.094. Erythromycin / Clindamycin resistance rates were 26.3% / 23.7% at BCCWH and 20.9% / 16.0% at LifeLabs. Inducible clindamycin resistance was 6.8% at BCCWH and 5.5% at LifeLabs.

Conclusions

GBS continue to be susceptible to penicillin. One community isolate had a penicillin MIC of 0.125 µg /mL. The high erythromycin and clindamycin resistance rates found are consistent with literature. Although resistance rates were higher at the hospital center, there were no statistically significant differences.

Vincent Fung

Vincent Fung[1], David Kendler[2]

[1]Department Medical Biochemistry, Pathology and Laboratory Medicine, University of British Columbia; [2]Department of Medicine, University of British Columbia

THE IMPACT OF INDIVIDUAL CLINICAL RISK FACTORS IN PREDICTING 10-YEAR FRACTURE RISK IN CANADIAN WOMEN OF VARIOUS AGE GROUPS USING FRACTURE RISK ASSESSMENT TOOL (FRAX)

Background/objectives

Recent Canadian osteoporosis guidelines have recommended the use of a 10-year fracture risk assessment tool such as FRAX as a way to determine fracture risk in their patients and to decide when to initiate pharmacological treatment. FRAX uses 12 risk factors to determine fracture risk. The objective of this study is to calculate the10-year probabilities of major osteoporotis-related fracture and hip fracture in hypothetical female patients with osteopenia between 40 and 80 years in Canada. It is recommended that this tool be used in cases when clinical or densitometric osteoporosis cannot be determined and a decision as to whether to treat requires such a tool Our analyses help the practitioner to understand the impact of risk factors on fracture risk estimates.

Methods

Using a hypothetical female patient with specified weight (67kg), height (165cm) and bone mineral density (T-score of -2.2), the 10-year probabilities of major osteoporotic-related and hip fracture across various age groups (40-80) are calculated by inputting the presence of individual clinical risk factors into the Canadian FRAX model (*http://www.sheffield.ac.uk/FRAX/tool.jsp?country=19*),

Results

Age alone (40 years to 80 years) increases 10-year probability of major-osteoporotic fracture from 2.4% to16% and hip fracture from 0.6% to 5%. The strongest clinical risk factors in predicting 10-year probability of major-osteoporotic fracture are glucocorticoid use, history of previous fracture and parental hip fracture from 40-80 years old. Smoking history, glucocorticoid use and history of previous fracture are the strongest predictive factors for hip fracture in women between 40-60 years old. After age 60, parental hip fracture becomes an important risk factor.

Conclusions

FRAX is a very useful tool to aid clinicians in the management of patients at risk of osteoporotic fracture. Aside from age, personal fracture history, glucocorticoid use, parental hip fracture and smoking history are the most important risk factors in predicting future osteoporotic fracture in a woman with normal BMI (24.6g/m2) and osteopenia (BMD: T-score of -2.2).

Tony L Ng[1,2], Gabriel Leprivier[1], Matthew D Robertson[1], Matthew J Martin[1], Keith R Laderoute[3], Elai Davicioni[4], Timothy J Triche[5], Poul HB Sorensen[1,2]

[1]Dept of Molecular Oncology, BC Cancer Research Centre; [2]Dept of Pathology and Laboratory Medicine, UBC; [3]Biosciences Division, SRI International; [4]GenomeDx Biosciences Inc; [5]Dept of Pathology, Children's Hospital Los Angeles

Tony Ng

THE AMPK STRESS RESPONSE PATHWAY MEDIATES ANOIKIS RESISTANCE THROUGH INHIBITION OF MTOR AND SUPPRESSION OF PROTEIN SYNTHESIS

Background/objectives

Anoikis is the physiologic response of non-hematopoetic cells to detachment from the extracellular matrix (ECM), in which detached cells undergo rapid apoptosis. Conversely, suppression of anoikis is a hallmark of cancer cells that allows them to survive in locations absent of ECM such as the lymphovascular space, likely representing an early and critical step in metastasis. Here, we wished to identify novel pathways in anoikis suppression.

Methods

Cell lines stably transformed with oncogenes known to robustly suppress anoikis, namely activated K-Ras(V12) and the fusion oncogene ETV6-NTRK3 (EN), were subjected to forced detachment versus standard monolayer conditions, analyzed by gene expression profiling, and pathways identified were characterized biochemically. The results were validated using breast cancer cell lines.

Results

A Gene expression profiling demonstrated that, following detachment, transformed cells induce multiple stress response pathways. After detachment, mouse embryo fibroblasts (MEFs) transformed by K-Ras(V12) or ETV6-NTRK3 (EN) activate a transcriptional response overrepresented by genes related to bioenergetic stress and the AMP-activated protein kinase (AMPK) energy-sensing pathway. Accordingly, AMPK is activated in both transformed and non-transformed cells following detachment and AMPK deficiency restores anoikis to transformed MEFs. However, AMPK activation represses the mTORC1 pathway only in transformed cells, suggesting a key role for AMPK-mediated mTORC1 inhibition in suppression of anoikis. Consistent with this, AMPK-/- MEFs transformed by EN or K-Ras show sustained mTORC1 activation following detachment and fail to suppress anoikis. Transformed TSC1-/- MEFs, which are incapable of suppressing mTORC1, also undergo anoikis following detachment which is reversed by mTORC1 inhibitors. We also explored how mTORC1 inhibition might be important for anoikis resistance. Transformed AMPK-/- and TSC1-/- MEFs have higher total protein translation levels compared to corresponding AMPK+/+ cells, and translation inhibition using cycloheximide partially restores anoikis resistance to these cells. Furthermore, breast cancer cell lines also show AMPK activation and mTORC1 inhibition following detachment, and AMPK inhibition in these cells promotes restoration of anoikis.

Conclusions

Our data implicate AMPK-mediated mTORC1 inhibition and suppression of protein synthesis as a means for bioenergetic conservation during detachment, thus promoting anoikis resistance.

Dmitry Turbin

D. Turbin, A. Chan, S. Leung, D. Thomson, B. Gilks, D. Huntsman, T. Nielsen

University of British Columbia; Genetic Pathology Evaluation Centre at Vancouver Coastal Health Research Insitute; British Columbia Cancer Agency; Vancouver, BC

A COMPUTER PROGRAM FOR NUCLEAR CHROMATIN ANALYSIS

Background/objectives

Visual assessment of nuclear chromatin is an essential component of microscopic diagnosis. Visual characteristics of chromatin (fine, coarse, open, "salt and pepper", etc.) are well described in the anatomical pathology literature and are important for differential diagnosis and grading of human neoplasms.

Methods

We developed a Java-based plugin for a widely-used open source image analysis program, ImageJ. Segmentation of nuclei is performed using intrinsic capabilities of ImageJ, including automatic or manually adjustable thresholding, morphological functions, and a watershed algorithm. Analysis of the chromatin structure of individual nuclei is then performed by our plugin, utilizing 21 Haralick's texture parameters. Results of the computations are saved in a tab-delimited format and can be used in statistical software for discriminant function calculations. We validated our program by analyzing the texture parameter of lymphocyte nuclei in a benign inflammatory infiltrate, and nuclei of invasive lobular breast carcinoma. These tissues were chosen because of their otherwise similar size and shape of nuclei, that make them challenging to distinguish for most of image analysis programs. The discriminant constant and discriminant coefficients were generated in SPSS 11.0 for Windows, allowing calculation of a single texture score for a given nucleus.

Results

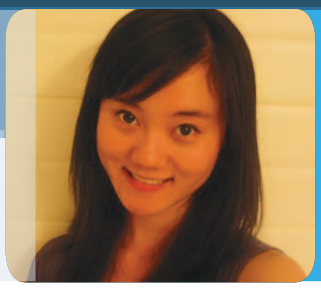
The program was trained on a set of 336 lymphocyte and 336 carcinoma nuclei, and another set with the same numbers of nuclei was used to validate differences in chromatin structure in these two tissues. A discriminant function value was calculated and used to differentiate between the nuclei of these two types of cells. The program was able to recognize 297 nuclei of lobular breast carcinoma cells (88.4%) based on the chromatin features alone, picking up 0 (0%) of nuclei of lymphocytes.

Conclusions

Differences between nuclei of benign and dysplastic / malignant cells of the same origin are extensively used in everyday pathology practice. We developed an image analysis program capable of nuclear chromatin texture quantitation using digital images of hematoxylin and eosin stained histological sections, analyzing 21 texture parameters. This program can work as a part of image analysis software or as a standalone program. To our knowledge, this is the only program that is able to analyze texture of individual objects within an image; the other known texture analysis programs use the whole image only. Numerical expression of the chromatin features can help in understanding tumour biology, in differential diagnosis, and performing automated image analysis.

Vivien Wei[2], Hong Xu[1], Rebecca Tong[2], Steven S.S. Poon[1], Steven McKinney[1] and Samuel Aparicio[1]

[1]Department of Molecular Oncology, BC Cancer Research Center, Vancouver, British Columbia, Canada; [2]University of British Columbia, Vancouver, Canada



Vivien Wei

CLONOGENIC ANALYSIS OF GENES THAT ARE SYNTHETICALLY LETHAL WITH BREAST CANCER 2 GENE (BRCA2)

Background/objectives

The concept of synthetic lethality describes the combination of mutation in two genes will lead to cell death while either alone is still compatible with cell viability. This paradigm provides a novel approach in the context of anti-cancer therapy. Targeting genes that are synthetically lethal with cancer-related mutations should kill cancer cells only. Breast Cancer 2 (BRCA2) gene is a tumor suppressor gene that is often defective in breast cancers. This gene has an important function in repairing DNA Double-Strand Breaks (DSBs). These breaks can be caused by endogenous means or exogenous damaging agents and are detrimental to the organism if left unrepaired. BRCA2 localize at the site of the break along with other essential proteins to repair damage through Homologous Recombination (HR) pathway. BRCA2 thus plays a critical role in maintaining genomic stability by preventing dangerous DNA rearrangements that could lead to pathological DNA structures - a hallmark of cancer. In this study, we hypothesize that BRCA2's synthetic lethal partners would be potential drug targets to selectively kill BRCA2 null function tumor cells. For this purpose, we used clonogenic assay to test a number of possible genetic interactions between BRCA2 and some genes identified from a genome-wide BRCA2 synthetic lethality screen.

Methods

HCT116 wild type and BRCA2-/- cells were grown in 10 cm tissue culture plates until subconfluence. Genes are knocked down using siRNA transfection (non-target siRNA is used as negative control). Cells are left to grow for 2 days then plated in fixed numbers into 6 cm plates in media containing increasing concentration of phleomycin. Plates are incubated for 10 days and then fixed and stained. Colony numbers are scored and survival fractions are compared.

Results

A Clonogenic analysis showed that DCP2, EXOSC5 and LSM7 has synthetic lethal interaction with BRCA2. This synthetic lethality has been confirmed by siRNA mediated transcription repression with both pooled siRNA and individual siRNA. We also analyze the possible mechanism of the synthetical interaction by testing the DNA damage drug sensitivity on the genes that interact genetically with BRCA2. DCP2-pooled knockdown showed a mild increase in DNA damage sensitivity compared to non-target control. EXOSC5-pooled and LSM7-pooled knockdowns had significant decrease in cell survival fraction compared to non-target control. These results indicate that the genes that are synthetically lethal with BRCA2 are possibly involved in DNA damage repair.

Conclusions

DCP2, EXOSC5 and LSM7 are synthetically lethal with BRCA2-/- and the possible reason for this interaction is the increased genome instability in BRCA2 knockout cells.

Tawny Hung[1], Rossitza Lazova[2]

[1]Department of Pathology, VGH; [2]Department of Dermatology, Yale University

Tawny Hung

INCREASED MITOTIC RATE AND KI67 EXPRESSION IN ULCERATED MELANOCYTIC NEVI

Background/objectives

We studied the prevalence of mitoses in ulcerated and non-ulcerated intradermal melanocytic nevi and compared the Ki67 expression and distribution in the two groups. 82 ulcerated and 98 non-ulcerated intradermal nevi were examined by H&E and studied for Ki67 expression. The number of Ki67-positive cells per mm2 and the mitotic index were recorded. 14 out of 82 ulcerated nevi (17%) showed mitotic figures on H&E examination whereas only 3 out of 98 (3%) non-ulcerated nevi harbored mitoses. Ki67 expression ranged from 3-60 per mm2 (mean 19) and from 2-18 cells per mm2 (mean 12) in the ulcerated and non-ulcerated nevi respectively. The mitotic index was less than 1% in both groups. The mitoses and Ki67 positive cells in the ulcerated nevi were distributed mostly around the ulcer base and in the adjacent superficial dermis. Ulcerated (traumatized) nevi show significantly higher number of mitoses and Ki67 positive cells (p= 0.0009) in comparison to non-traumatized nevi, in which mitoses are rare.

Paul R. Hiebert[1,2], Wendy A. Boivin[1,2], Thomas Abraham[1], Sara Pazooki[1], Hongyan Zhao[1], David J. Granville[1,2]

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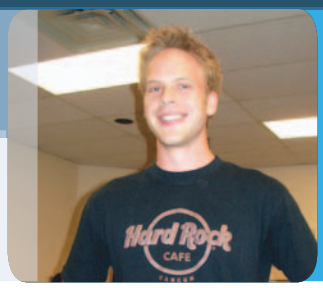


Paul Hiebert

GRANZYME B CONTRIBUTES TO EXTRACELLULAR MATRIX REMODELING AND SKIN AGING IN APOLIPOPROTEIN E KNOCKOUT MICE

Background/objectives

Apolipoprotein E (apoE) is an important mediator of circulating cholesterol and is vital for proper cholesterol clearance. In addition, apoE is expressed in the skin and has important anti-inflammatory properties. ApoE knockout (apoE-KO) mice therefore display skin that is chronically inflamed and demonstrate signs of accelerated aging such as skin thinning and collagen remodeling, a phenotype that becomes even more pronounced when mice are fed a high fat diet. Granzyme B (GrB) is a serine protease expressed by immune cells and is involved in the induction of apoptosis in virally infected cells. During periods of chronic inflammation, GrB can be released into the extracellular environment where it can degrade the extracellular matrix (ECM) component decorin. Decorin is a proteoglycan that associates with collagen and is critical for proper collagen organization and structure. We therefore hypothesized that extracellular GrB activity was contributing to collagen remodeling in the chronically inflamed skin of apoE-KO mice through the cleavage of decorin.

Methods

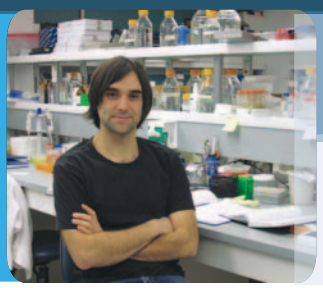
Thirty week old wild type (WT), apoE-KO and GrB/apoE double knockout (DKO) mouse skin was examined using hematoxylin & eosin, picrosirius red and luna's elastin stains to assess morphology, collagen and elastin respectively, in formalin fixed skin sections. Multi-photon microscopy was also used on fresh ex vivo skin samples to examine collagen organization and density. Immunohistochemistry was also performed on fixed skin sections to examine decorin and GrB.

Results

ApoE-KO mice fed a high fat diet demonstrated frailty, increased morbidity, skin thinning and a loss of dermal collagen density and organization. DKO mice showed protection against these deleterious changes. High fat diet fed apoE-KO mice also showed reduced decorin levels in the skin, while DKO mice showed increased decorin. GrB staining was also observed in the skin of apoE-KO mice, particularly in and around mast cells and areas of matrix remodeling.

Conclusions

ApoE-KO mice fed a high fat diet display features of premature aging skin, a phenotype that is reduced when knocking out GrB suggesting that GrB plays a role in skin aging and collagen disorganization, possibly through the cleavage of decorin. These results help in our understanding of possible mechanisms of aging skin and identify new potential therapeutic targets to treat patients suffering from premature aging or diseased skin.



Alon Hendel, David Granville

UBC James Hogg Research Centre, Institute for HEART+LUNG Health

Alon Hendel

ABSTRACT #14

GRANZYME B DISRUPTS ENDOTHELIAL CELL ADHESION TO FIBRONECTIN

Background/objectives

Granzyme B (GzmB) is a serine protease that is released by a variety of immune cells during chronic inflammation. GzmB accumulates extracellularly during chronic inflammation where it can cleave extracellular matrix (ECM) proteins. Although several studies have demonstrated GzmB-mediated ECM cleavage in vitro, the mechanism(s) by which extracellular GzmB contributes to disease progression remains poorly understood. The extracellular accumulation of GzmB is observed in a number of chronic inflammatory disorders where dysregulated angiogenesis contributes to disease progression. Work from our laboratory and others have identified several ECM proteins that are cleaved by GzmB in-vitro including, fibrinogen, fibronectin, vitronectin and decorin, all of which play a pivotal role in regulating angiogenesis. Specifically, fibronectin can modulate angiogenesis directly through its interaction with endothelial cells (ECs) or indirectly via the binding of pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF).

Hypothesis: As cleavage of fibronectin disrupts its ability to interact with EC and may induce VEGF release, we hypothesized that GzmB contributes to dysregulation of angiogenesis by cleaving fibronectin during inflammation.

Methods

Soluble fibronectin was incubated with GzmB and cleavage was examined by western blotting. Cleavage of fibronectin in its matrix form was evaluated by incubating fibronectin coated culture plates with GzmB and analysing the supernatant for detection of fibronectin released fragments by western blotting. The impact of GzmB-mediated fibronectin cleavage on EC adhesion and angiogenesis was evaluated using EC adhesion assays.

Results

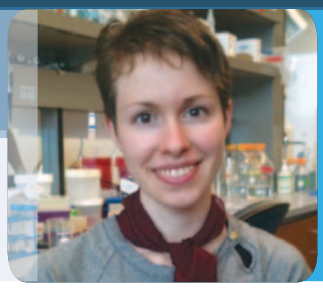
GzmB effectively cleaves soluble fibronectin and induces the release of fibronectin fragments from pre-coated plates. The release of fibronectin fragments by GzmB was dose dependent and was inhibited using a GzmB-specific inhibitor. GzmB-mediated fibronectin cleavage resulted in a significant, dose-dependent reduction in EC adhesion to fibronectin, which was restored by GzmB inhibition.

Conclusion

GzmB disrupts EC binding and adhesion to fibronectin. As EC interaction with fibronectin is essential for the induction of angiogenesis, GzmB-mediated cleavage of fibronectin may disrupt the ability of EC to form functional neovessels, leading to impaired angiogenesis.

Clara Y. Westwell-Roper[1], Derek L. Dai[1], Galina Soukhatcheva[1], Jan A. Ehses[2], C. Bruce Verchere[1,2]

[1]Department of Pathology and Laboratory Medicine, UBC; [2]Department of Surgery, UBC



Clara Westwell-Roper

BLOCKADE OF INTERLEUKIN-1 SIGNALLING IMPROVES HUMAN ISLET AMYLOID POLYPEPTIDE-INDUCED PANCREATIC ISLET GRAFT DYSFUNCTION

Background/objectives

Human islet amyloid polypeptide (hIAPP) is a 37-amino acid peptide co-secreted with insulin by pancreatic beta-cells. Pathological hIAPP aggregation occurs in type 2 diabetic and transplanted islets, both characterized by inflammation with macrophage infiltration. hIAPP aggregates share a common cross beta-sheet structure with other amyloids known to induce a potent pro-inflammatory response mediated by macrophage release of interleukin-1 (IL-1) beta. To determine whether hIAPP induces a similar pro-inflammatory response in transplanted islets, we evaluated the effect of IL-1 receptor antagonist (IL-1Ra) on hIAPP-induced islet graft dysfunction.

Methods

Donor islets were isolated from wild-type FVB mice or from littermates expressing hIAPP under the control of a beta-cell-specific promoter. Eleven-week-old male NOD/SCID recipients were rendered diabetic with streptozotocin, then implanted with a mini-osmotic pump containing IL-1Ra (50 mg/kg/d) or saline. One day following pump implantation, 150 islets were transplanted under the kidney capsule of recipient mice. After eight weeks, graft function was evaluated by intraperitoneal glucose tolerance testing. Amyloid deposition was assessed by Thioflavin-S staining of grafts and intra-graft macrophages were quantified by staining for the macrophage glycoprotein F4/80.

Results

Islet grafts expressing hIAPP contained Thioflavin-S-positive amyloid deposits in close association with F4/80-expressing macrophages. Transgenic grafts contained 50% more macrophages than wild-type grafts ($p<0.05$), an effect that was significantly inhibited by IL-1Ra. Furthermore, a three-fold reduction in amyloid area was observed in transgenic grafts from IL-1Ra-treated recipients, suggesting that hIAPP aggregation may not only contribute to IL-1 release but may also be a consequence of islet inflammation. Recipients of transgenic islet grafts displayed impaired glucose tolerance eight weeks following transplantation compared to recipients of wild-type grafts (area under the curve (AUC) = 1940±80 vs. 1260±60, $p<0.001$). Administration of IL-1Ra significantly improved graft function in recipients of transgenic grafts (AUC = 1470±160 vs. 1940±80, $p<0.005$) but not wild-type grafts, suggesting an important role for IL-1 in mediating hIAPP-induced islet inflammation and dysfunction.

Conclusions

hIAPP aggregation in transplanted islets promotes macrophage recruitment and islet dysfunction in an IL-1-dependent manner. Thus, anti-IL-1 therapy may improve graft function in human islet transplant recipients by inhibiting hIAPP-induced inflammation or attenuating amyloid formation. Importantly, these data point to a common mechanism of innate immune activation associated with diverse forms of amyloid disease.

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Loraine Bischoff

RECRUITMENT OF T REGULATORY CELLS TO ISLET GRAFTS PREVENTS RECURRENT AUTOIMMUNE DIABETES

Background/objectives

Type 1 diabetes is an autoimmune disease characterized by destruction of insulin-producing beta cells by autoreactive T cells. Islet transplantation represents a promising therapy for type 1 diabetes but is still challenged by the high rate of graft failure due to alloimmune and recurrent autoimmune responses. Given their ability to induce tolerance, CD4+ FoxP3+ T regulatory cells (Tregs) have great therapeutic potential in autoimmune diseases and transplantation. Some tumors recruit Tregs by secreting the chemokine CCL22 and thereby evade the immune response. In this study, we sought to overexpress CCL22 in islet grafts in order to attract Tregs to the transplant site and block recurrent autoimmunity in the non obese diabetic (NOD) mouse model of type 1 diabetes.

Methods

A recombinant adenoviral vector (Ad-CCL22) was created in which a mouse CCL22 cDNA is expressed downstream of the CMV promoter to induce constitutive secretion of CCL22. Islets from NOD.scid donors were isolated and incubated overnight with Ad-CCL22, Ad-LacZ or media alone (as controls) prior to transplantation into diabetic NOD recipients.

Results

In vitro experiments showed that an MOI of 10 was an appropriate dose to efficiently transduce islets with Ad-CCL22 without adversely affecting their function. CCL22-expressing islet grafts survived significantly longer (mean survival: 27.6± 7 days) compared to Ad-LacZ and untreated grafts, which all failed within 10 days ($p<0.01$). Histological analysis of islet grafts 10 days post-transplantation confirmed that insulin-producing cells were preserved in Ad-CCL22 but not in control grafts. Additionally, Ad-CCL22 grafts contained more FoxP3+ cells than control grafts despite similar immune infiltration. Flow cytometric analysis of grafts revealed a 7-fold increase ($p<0.001$) of CD4+FoxP3+ cells in CCL22-expressing grafts compared to Ad-LacZ transduced grafts. Moreover, depletion of CD4+ CD25+ cells in CCL22 transplant recipients abrogated the protective effect of CCL22 on islet graft survival.

Conclusion

These findings demonstrate that CCL22 expression in islets enhances influx of Tregs to the graft and protects from recurrent autoimmune diabetes. Our study shows that Treg trafficking can be manipulated to modulate the immune response in sites of inflammation and has implications for developing new therapies not only for transplantation but also for inflammatory and autoimmune diseases such as type 1 diabetes.

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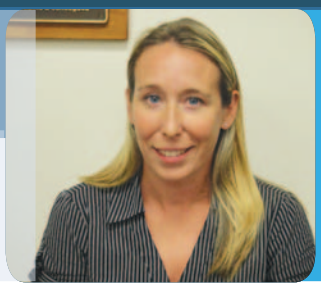

Melissa McConechy

IDENTIFICATION OF SUBTYPE-SPECIFIC SOMATIC MUTATIONS OF PPP2R1A IN ENDOMETRIAL AND OVARIAN CARCINOMAS

Background/objectives

The protein phosphatase 2, regulatory subunit A, alpha (PPP2R1A, or PR65a) gene is the scaffolding subunit of the serine-threonine protein phosphatase 2A (PP2A) holoenzyme. PP2A is involved in diverse cellular functions such as growth and survival cellular pathways, viral-induced cellular transformation, and has been suggested to play a tumour suppressor role in some human cancers. The PP2A holoenzyme is made up of the A scaffolding subunit (isoforms alpha and beta), the C catalytic subunit (alpha and beta) and multiple regulatory B subunits also with many isoforms. PPP2R1A is made up of 15 HEAT domains forming a horseshoe-like multi-helical structure that interacts with both the B and C subunits of PP2A. Somatic mutations in PPP2R1A have been found at low frequencies in lung, breast cancer and melanoma and most recently through whole-exome sequencing in ovarian clear cell carcinoma. We have examined whole-transcriptome sequencing data from ovarian clear cell carcinomas and identified a cluster of mutations in exon 5 of PPP2R1A. We further hypothesized that these mutations may also be present in endometrial carcinomas. We therefore examined the frequency of PPP2R1A mutations within exon 5 and 6 across the subtypes of both ovarian and endometrial carcinomas.

Methods

DNA from endometrial and ovarian subtypes were sequenced across exons 5 and 6 of PPP2R1A using PCR based Sanger sequencing, and somatic mutation status was verified by sequencing matched normal DNA.

Results

Targeted sequencing of PPP2R1A revealed somatic missense mutations in 40.8% (20 of 49) of endometrial high-grade serous tumours. Conversely, no mutations (0 of 50) were found in ovarian high-grade serous carcinoma cases. Mutations were also identified at a lower frequency of 5.0% (3 of 60) in endometrial endometrioid carcinomas, and within ovarian subtype tumours at 12.2% (5 of 41) in endometrioid, 4.1% (2 of 49) in clear cell, and 0% (0 of 12) of low-grade serous carcinomas.

Conclusions

We have identified a high frequency of subtype-specific PPP2R1A somatic mutations within endometrial high-grade serous carcinoma, but not in high-grade serous carcinoma of the ovary. Importantly, this study provides the first molecular evidence that endometrial and ovarian high-grade serous carcinomas may arise from different molecular genetic pathways. Theses mutations result in the modification of highly conserved amino acid residues known to be important interaction sites with regulatory B subunits. These mutations likely play a role in destabilization of the PP2A complex by inhibiting interactions with B subunits, and a probable role in cellular tumourgenesis. A closer survey of mutations found within PPP2R1A and the PP2A complex, as well as functional characterization of this pathway, may pave the way to defining biologically relevant pathways and targets for high-grade serous endometrial carcinomas, ultimately leading to improved patient-targeted therapeutics.

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Jennifer Choo

AN IMMUNOHISTOCHEMICAL SURVEY OF BIOMARKERS FOR BASAL-LIKE BREAST CANCER AGAINST A GENE EXPRESSION PROFILE GOLD STANDARD

ABSTRACT #18

Background/objectives

Gene expression profiling of breast cancer delineates a particularly aggressive subtype referred to as "basal-like," which comprises ~15% of all breast cancers, afflicts younger women and is refractory to endocrine and anti-HER2 therapies. Immunohistochemical surrogate definitions for basal-like breast cancer, such as the clinical ER/PR/HER2 triple negative phenotype and models incorporating positive expression for CK5 and/or EGFR are widely employed. However, many additional biomarkers for basal-like breast cancer have been described. The objective of the current study was to perform a comprehensive evaluation of proposed biomarkers to determine how to best define basal-like breast cancer in a clinical setting.

Methods

71 proposed biomarkers of basal-like breast cancer were drawn from gene expression profile data and literature review. 62 of 71 biomarkers underwent immunohistochemical optimization. 45 were successfully optimized and evaluated on a breast cancer tissue microarray containing 122 cases with accompanying gene expression profile data for gold standard subtype assignment.

Results

25 of 45 biomarkers were significantly associated with the basal-like subtype after correction for multiple comparisons. Ki67 and PPH3 were the most sensitive biomarkers (both 94%), whereas CK14, IMP3 and NGFR were the most specific (100%). Nestin (OR = 28.7, 55% sensitivity, 95% specificity) was the most accurate single biomarker. Based on published precedents, provisional model building on the current dataset found that a panel consisting of ER/HER2 double negativity plus positivity of nestin, CK5 or fascin possesses a greater level of agreement (kappa = 0.75) with gene expression profiling than either ER/PR/HER2 triple negative (kappa = 0.65) or the previously-published surrogate panel including CK5 and/or EGFR positivity (kappa = 0.66).

Conclusion

Optimized immunohistochemical tests for basal-like breast cancer are easily applied to paraffin-embedded tissue specimens. Subject to data reduction and statistical model building techniques coupled with validation on an independent series, this comprehensive immunohistochemical survey can be used to build an optimal immunopanel that best defines basal-like breast cancer in a clinical setting. Application to retrospective analyses and prospective clinical trials would facilitate development of therapies for this particularly aggressive and deadly form of breast cancer.

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Amal El-Naggar

INSIGHTS INTO THE ROLE OF THE Y-BOX BINDING PROTEIN-1 (YB-1) IN SARCOMAS

Background/objectives

Sarcomas are a diverse group of malignant neoplasms of which are characterized by early metastatic spread, aggressive behavior, and poor prognosis. One of the genes believed to play a role in sarcomatogenesis is the Y-box binding protein-1 (YB-1). YB-1 is a member of the highly conserved heat shock domain-containing family of proteins known to bind single and double stranded DNA and single stranded RNA, thereby controlling transcription and translation of a multitude of genes. Our previous studies using a breast cancer model showed that enforced expression of YB-1 promotes an epithelial-mesenchymal transition (EMT) in non-invasive breast epithelial cells, through downregulation of epithelial- and growth-related genes and simultaneous activation of mesenchymal genes. YB-1 induced EMT was accompanied by an enhanced metastatic potential and reduced proliferation rates. In accordance, YB-1 was shown to be upregulated in invasive breast cancer cells and correlated with poor survival. Studies that comprehensively investigate the role of YB-1 in sarcomas development are currently lacking.

Objective: To identify the potential roles played by YB-1 in sarcomas development and to determine whether YB-1 can serve as a therapeutic target for sarcomas.

Aim: Identify the effect of YB-1 knockdown on the morphology, growth, migration, and invasion of sarcoma cell lines.

Methods

Rh30 (rhabdomyosarcoma), MG63 (osteosarcoma), and TC32 (Ewing family tumors) cell lines were chosen as models for the current study. The cells were treated with siCTRL or siYB-1 siRNAs. Sarcoma cell morphology was assessed using phase-contrast microscopy. Cell transformation was assessed using western blotting, soft agar colony formation assays and growth curves. Cell motility was assessed 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 HIF1a and their ability to promote metastasis.

Results

Inhibiting YB-1 expression using siRNAs led to marked changes in sarcoma cell morphology resulting in: 1) loss of spindle-shaped morphology, 2) a significant increase in cell proliferation and 3) a significant reduction in cell motility. Our results are in concordance with what we observed in our previous breast model studies. We also found that under hypoxic conditions, both YB-1 and HIF1a were induced. Using Polysomal fractionation and qPCR, we determined that YB-1 is a major translational regulator of HIF1a. These findings may provide a mechanism to help explain how YB-1 p

Conclusions

1- YB-1 is essential for establishing sarcoma cell morphology.

2- YB-1 is a major regulator of sarcoma cell motility at the expense of cell growth.

3- The aggressiveness of sarcoma cells may be attributed to YB-1 and its downstream mediator HIF-1a.

4- YB-1 may represent a promising future target in treatment of sarcomas.

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Yu Chi (Kevin) Yang

SMALL MOLECULES TO INHIBIT PROSTATE CANCER PROLIFERATION BY TARGETING ANDROGEN RECEPTOR

Background/objectives

Prostate cancer (PCa) is the most frequently diagnosed cancer and the second leading cause of cancer death in North American men. Localized disease can be effectively managed by surgery and radiation, but unfortunately all currently available treatments including androgen ablation therapy, are only palliative for advanced PCa and fail to save lives. Therefore it is imperative to continue the search for effective therapeutic agents. In this study, the objectives are to investigate the effect of small molecule Terpenes (T1 and T2) on the proliferation of prostate cancer cells, and provide further characterization of the Terpenes if antiproliferative activities are observed.

Methods

Prostate cancer cell lines, including androgen-sensitive LNCaP and androgen-insensitive PC3, were treated with T1 or T2 at 10 μM to evaluate the effect on cellular proliferation by quantifying BrdU incorporation. To measure AR transcriptional activity, AR-driven reporters were transiently transfected into LNCaP cells, which were subsequently treated with 10 μM T1 or T2 with or without androgen. 48 hours after treatment, cells were harvested to measure the reporter activity, which was normalized to protein concentration. In addition, to see if blockage of AR nuclear translocation was a possible mechanism for inhibition, AR-YFP was transfected into LNCaP cells and treated with 10 μM T1 or T2. Steroid receptor specificity was examined by measuring the transcriptional activities of progesterone receptor (PR) and glucocorticoid receptor (GR). Finally, in vitro ligand-binding competition assay was employed to confirm the binding of the Terpenes to AR ligand-binding domain (LBD).

Results

T1 and T2 are identical except the presence of two double bonds. While T2 had no significant biological activities, it was showed that T1: (1) inhibited androgen-dependent proliferation without affecting cells that are not dependent on AR for proliferation; (2) blocked AR transcriptional activity induced by androgen; (3) did not prevent the androgen-induced nuclear translocation of AR; (4) demonstrated steroid receptor specificity by showing no effect on GR transcriptional activity; and (5) interacted with AR-LBD by weak ligand binding affinity.

Conclusion

T1 has been shown to be a potent inhibitor of AR transcriptional activity without abolishing AR nuclear translocation. By blocking AR, T1 significantly attenuated androgen-dependent proliferation in prostate cancer cells. Furthermore, the structure-activity relationship between T1 and T2 could provide crucial insight for the development of drugs to treat prostate cancer.

POST-DOCTORAL FELLOW

Maged Gomaa Hemida, Xin Ye, Mary Zhang, Paul J Hanson, Bruce McManus, and Decheng Yang

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Maged Hemida

MicroRNA-203 PROMOTES COXSACKIEVIRUS B3 REPLICATION THROUGH TARGETING THE ZINC FINGER PROTEIN 148

Background/objectives

Coxsackievirus B3 (CVB3) is one of the most common causes of viral myocarditis. Previous studies found that CVB3 infection depending on its receptor expression. However, further studies found that the expression levels of the receptor in different tissues of mouse do not always correlate well with the infectivity of CVB3 in the corresponding organs, which raises the possibility that other factors within the host cells may also play an important role in determining the viral tissue tropism and pathogenesis. MicroRNAs (miRNA) are post-transcriptional gene regulators and they are predicted to regulate at least one third of human genes. Although CVB3 does not encode any miRNAs, it may modulate the host miRNAs to benefit its replication. Bioinformatics data suggest that miR-203 can target zinc finger protein-148 (ZFP-148), a transcription factor involved in regulating the cell growth arrest factor p21. The goal of this study is to examine the role of the miR-203 in regulating CVB3 replication.

Methods

Microarray analysis was employed to detect changes in the miRNA expression profile in CVB3 infected A/J mice hearts at day 4 and 7 post-infection (dpi). Q-RT-PCR was conducted to confirm the results of the Microarray data. To evaluate ZFP-148 expression and CVB3 replication efficiency, supernatants and cell lysates were collected from HeLa cells transfected with pre-miR-203 and analyzed by Western blot, plaque assay, and qRT-PCR. PmirGLO dual luciferase assay was conducted by cotransfection of HeLa cells with ZFP-148 3'UTR construct and the miR-203 or control miRNA to validate ZFP-148 as a novel target for the miR-203. siRNA specific to ZFP-148 was transfected into CVB3-infected cells to examine the effect of downregulation of ZFP-148 on CVB3 replication.

Results

Microarray analysis demonstrated that miRNA-203 was significantly upregulated in CVB3-infected mouse hearts and this data was further confirmed by qRT-PCR. In functional study of this miRNA in regulating CVB3 infectivity, we found that miR-203 expression enhancedCVB3 replication in miRNA-203-transfected HeLa cells. Further examination of the mechanism revealed that miR-203 promotedCVB3 replication through targeting zinc finger protein gene-148 (ZFP-148) and subsequently promoting host cell growth by inhibiting p21, a cell cycle arrest kinase inhibitor, and by up-regulating Bcl-XL, an anti-apoptotic protein. Finally, we further confirmed the specific targeting of miRNA-203 to ZFP-148 by luciferase assay using wild-type and mutant reporter constructs.

Conclusions

Taken together, these data suggest that miRNA expression profile is a molecular determinant of host cell susceptibility toCVB3 infection. The up-regulated miR-203 plays an important role in enhancingCVB3 replication through targeting ZFP-148 and subsequently promoting host cell growth.

Edwin Gershom

Edwin S Gershom, Amanda Vanden Hoek, Michael R Sutherland, and Ed L.G. Pryzdial

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HERPESVIRUSES ENHANCE FIBRINOGEN CLOT LYSIS

Background/objectives

Herpesviruses have been implicated in vascular disease. To explain the correlation on a molecular basis we have shown that the virus envelope contains anionic phospholipid derived from host cells, and proteins encoded by the host (tissue factor) as well as the viral (glycoprotein C) genomes, which initiate blood coagulation. Thus, the virus infection should be a strong independent predictor of vascular disease. Nevertheless, the clinical correlation is relatively weak, becoming more significant in combination with other risk factors. To explain this discrepancy, the current work is based on our additional report that at least one Herpesvirus (cytomegalovirus (CMV)) has host-genome-encoded annexin II (A2) on its surface. A2 is known to accelerate tissue plasminogen activator (tPA)-mediated activation of plasminogen (Pg) to plasmin (Pn) because of C-terminal lysines that interact with both Pg and tPA. Pn is the primary fibrinolytic protease, necessary for physiological clot dissolution. Thus, these viruses may facilitate clearance of the fibrin they generate.

Hypothesis: Herpesviruses enhance tPA-mediated Pn generation, correlating to virus associated A2.

Methods

Purified herpes simplex virus type 1 (HSV1) and 2 (HSV2) and CMV were quantified by electron microscopy. Cellular A2 expression varies, therefore HSV1 was propagated in several cell lines. Using purified Pg and tPA, virus-mediated Pn generation was followed in a chromogenic assay. The contribution of viruses to fibrin clot lysis using purified proteins was investigated by light scattering. Pg-conjugated horse radish peroxidase (Pg-HRP) and western blots were used to identify Pg-binding species and A2 associated with the virus, respectively. The effect of Pn-mediated signalling on virus infection was determined using cytopathic plaque assays.

Results

Chromogenic experiments demonstrated that HSV1, HSV2 and CMV enhanced Pg activation in a dose-dependent manner by up to 5-fold, regardless of the parental cell line. Prolonged incubation confirmed the requirement for exogenous tPA. Pg-HRP bound to a number of virus-associated proteins and was shown to be C-terminal lysine-dependent by complete inhibition with epsilon-aminocaproic acid (EACA). A2 was demonstrated to be associated with purified HSV1 cultured in different cells except when propagated in an A2 negative cell line. An A2 antibody inhibited binding of Pg-HRP to viral A2. HSV1, HSV2 and CMV accelerated fibrin clot lysis, which was inhibited in the presence of EACA and aprotinin, Pn inhibitor. Unlike the chromogenic assay of Pn generation, each virus also exhibited a clot lysis mechanism independent of added tPA. As previously identified for thrombin, incubation of host cells with purified Pn enhanced virus infection by over 3-fold.

Conclusion

HSV1, HSV2 and CMV accelerate tPA-mediated Pn generation in the absence of fibrin and identify A2 as one of several Pg binding partners. Furthermore, purified HSV1, HSV2 and CMV facilitating fibrin clot dissolution in the absence of exogenous tPA, is novel. The mechanism is not clear, but requires Pg activation. Purified Pn enhancing infection suggests why these viruses initiate Pn generation. Hence, the virus-mediated activation of fibrinolysis may compensate its its ability to trigger coagulation and attenuate potential risk as an independent predictor of vascular disease.

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Hayley Spencer

INVESTIGATION OF ULTRASTRUCTURAL CHANGES IN THE LIVER OF HEPATITIS C VIRUS AND HIV CO-INFECTED PATIENTS UNDERGOING HCV THERAPY

Background/objectives

End-stage liver disease is now the leading cause of death for patients infected with HIV in the developed world. In Vancouver, approximately 50% of HIV-infected individuals are co-infected with hepatitis C virus (HCV), leading to accelerated liver disease progression. Although HCV therapy is beneficial to the liver, both viruses, as well as their respective treatments: highly active antiretroviral therapy (HAART) and HCV antiviral therapy can promote liver damage and toxicity, and potentially contribute to liver failure. In particular, nucleoside analogues, a drug class used in both HIV and HCV therapy can exert mitochondrial toxicity.

Objective: To investigate changes in liver ultrastructure and mitochondrial DNA (mtDNA) quantity and quality following HCV antiviral therapy in co-infected individuals, whether on or off-HAART. We hypothesize that HCV treatment will be associated with changes in hepatocyte ultrastructure consistent with either further damage or cellular repair.

Methods

Two pairs of liver biopsies were collected from co-infected study participants (N=11 and still accruing): one before and one after HCV antiviral therapy. Each time, one biopsy was processed for light microscopy for Pathologist's scoring of fibrosis and inflammation, the only microscopic assessment of liver damage performed as part of standard clinical care. Aperio images of the slides were used to determine the degree of hepatocyte binucleation. The other biopsy was processed for TEM and a portion kept for nucleic acid analyses. TEM images were used to estimate hepatocyte cell volume, as well as mitochondria, lysosome, glycogen and lipid volume fractions. All analyses are conducted in a random and blinded fashion.

Preliminary Results

Although still blinded, after the characterization of half the biopsies, a wide spectrum has been observed with respect to qualitative observations such as large tertiary lysosomes, dysmorphic mitochondria, glycogen density and distribution, and lipid content. Representative examples will be presented.

Conclusions

Ongoing experiments: mtDNA content is being analysed by qPCR, mtDNA deletions by long-PCR, while mtDNA oxidative damage will be assessed using a long-PCR/qPCR-based assay. Before and after HCV therapy samples will be compared, and related to HCV therapy outcome, biopsy pathology scores, patient HIV therapy and clinical blood work.

Dian Sulistyoningrum

Dian C Sulistyoningrum[1], Timothy J Green[3], Scott A Lear[4], Angela M Devlin[2]

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ABSTRACT #24

ETHNICITY, ADIPOSITY AND CARDIOVASCULAR RISK FACTORS ARE ASSOCIATED WITH VITAMIN D STATUS

Background/objectives

Previous studies have shown that vitamin D deficiency, a risk factor for cardiovascular disease, is prevalent in obese individuals. Vitamin D status is dependent on sun exposure, age, sex, skin color, and body fat distribution. The Multicultural-Community

Health Assessment Trial (M-CHAT) showed that ethnicity predicts body fat distribution with greater visceral adipose tissue (VAT) per kg of body fat in South Asians compared to Europeans. The goal of this study is to investigate if serum 25-hydroxyvitamin D3 [25(OH)D] is associated with body fat deposition.

Methods

Study participants were part of the M-CHAT, which is a multi-ethnic population study that includes Aboriginal, Chinese, European, and South Asian individuals residing in Vancouver, BC. A total of 187 European and 192 South Asian individuals were included in this study. Body mass index (BMI), waist circumference, fasting plasma samples for high-density lipoprotein (HDL) cholesterol, triglycerides, glucose and insulin levels were measured, as well as homeostatic model assessment-insulin resistance (HOMA-IR). VAT was quantified by CT scanner. Total abdominal adipose tissue (TAT) was calculated from the adipose tissue originated within the inside edge of the abdominal wall. Subcutaneous adipose tissue (SAT) was calculated as the difference between TAT and VAT. Total body fat was quantified by dual-energy x-ray absorptiometry (DEXA). Serum 25(OH)D was quantified by competitive chemiluminescence immunoassay. Participants' whole blood DNA was genotyped for vitamin D binding protein (GC) protein rs2282679 variant. Linear regression analysis was used to assess the association between serum 25(OH)D and clinical and metabolic parameters. Models were adjusted for age, ethnicity, sex, smoking and season of blood collection.

Results

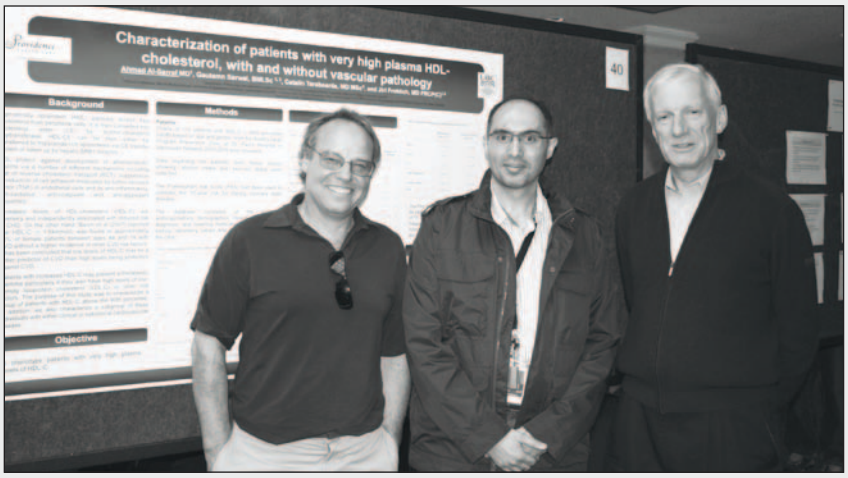
South Asians had lower ($P<0.001$) levels of 25(OH)D than Europeans. Using regression models adjusted for ethnicity, 25(OH)D was inversely associated with season, percent body HOMA-IR and age ($P< 0.05$). Following further adjustment for body fat distribution serum

25(OH)D was inversely associated with VAT ($P<0.001$) but not subcutaneous adipose tissue (SAT). When VAT and SAT were included in the same model, the relationship of serum 25(OH)D with VAT remained ($P = 0.001$) and there was no relationship with SAT. Similar results were also found when Europeans and South Asians were analyzed separately. We saw a trend towards lower serum 25(OH)D in individuals carrying the minor G allele of the GC protein rs2282679 variant in both European and South Asian subjects.

Conclusion

These findings show that vitamin D levels are strongly associated with VAT deposition and are lower in South Asians further contributing to the elevated risk for cardiovascular disease in this population. The association also suggests that poor vitamin D status may play a role in the pathology of CVD associated with VAT, and warrants further investigation.

Poster Presentations



Pathology Conference is a day when we get to hear about the exciting research projects in which our Residents, Graduate Students, Postdoctoral Fellows, and others are involved. The poster session gives the

opportunity for all Department members and guests to interact with these young researchers.

This is the next generation of scientists who will undoubtedly make significant contributions to both health care and to the new knowledge that will continue to make improvements in our ability to deal with disease and create healthier populations. For that reason, they deserve to be recognized on occasions such as this.

May , 2011

Melissa Glier

MB Glier[1], D Sulistyoningrum[1], E Wang[2], S Ghosh[1] and AM Devlin[1,2]

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CARDIAC LIPOTOXICITY IS GREATER IN CYSTATHIONINE-β-SYNTHASE DEFICIENT MICE WITH DIET INDUCED OBESITY

Background/objectives

Obesity is a common cardiovascular disease (CVD) risk factor. Obesity-related cardiac lipid accumulation is associated with oxidative stress and cardiac dysfunction. The roles of the antioxidant glutathione and the factors controlling its homeostasis in cardiac lipotoxicity are unclear. A large body of evidence suggest that the liver is the main source for glutathione synthesis. The enzyme Cystathionine-beta-synthase (CBS) catalyzes the first step in the transsulfuration of homocysteine to cysteine and provides 50% of the total cysteine required for liver glutathione synthesis. To date, it is thought that liver levels of glutathione are reflective of glutathione levels in other tissues. Therefore, disturbances in liver glutathione synthesis could have profound consequences of glutathione concentrations in other organs such as the heart, impairing its ability to combat oxidative stress.

Objective: Based on the previous evidence, we hypothesize that mice heterozygous for targeted disruption of Cbs (Cbs +/-) will be more susceptible to obesity-related cardiac lipotoxicity because of impaired synthesis of liver glutathione.

Methods

Young adult C57BL/6J (Cbs +/+) mice and mice heterozygous for targeted disruption of the gene for Cbs (Cbs +/-) were fed either a chow diet (12% energy from fat) or a high fat diet (HFD) (60% energy from fat) from weaning for 13 weeks to induce obesity. At 15 weeks of age, mice were subjected to a glucose tolerance test (2g/kg of body weight). Tissue and plasma triglyceride levels were quantified by HPLC-MS/MS. Glutathione, insulin and glucose were quantified using commercial kits. Protein levels were quantified by immunoblot.

Results

Cbs +/+ and Cbs +/- mice fed a HFD had similar body weight gain ($P<0.01$), visceral ($P<0.01$) and subcutaneous ($P<0.01$) adiposity as well as increases in liver triglyceride levels ($P<0.01$). No differences in liver total glutathione or plasma triglycerides were found. However, Cbs +/- mice fed the HFD had higher fasting glucose levels ($P<0.01$) at baseline and greater glucose intolerance ($P<0.01$) than Cbs +/+ mice fed the HFD. Furthermore, both Cbs +/+ and Cbs +/- mice fed a HFD had similar increases in fasting insulin levels ($P<0.01$) at baseline than mice fed a chow diet. Interestingly, Cbs +/- mice have lower levels of heart total and reduced GSH ($P<0.01$) than Cbs +/+ mice and this is accompanied by higher heart triglyceride levels ($P< 0.01$) with the greatest levels ($P< 0.01$) observed in Cbs +/- mice fed the HFD. This was accompanied by greater heart protein markers of oxidative stress (lipid hydroperoxides and nitrotyrosines, $P<0.01$); mitochondria biogenesis (peroxisome proliferator activated receptor γ co-activator 1 alpha, $P<0.01$); and apoptosis (caspase-3 and X-linked inhibitor of apoptosis, $P<0.01$).

Conclusion

These data provides an integrated view of hepatic and cardiac glutathione homeostasis during lipotoxicity and demonstrates a novel role for Cbs in maintaining cardiac glutathione, and protecting against cardiac lipid accumulation and oxidative damage during obesity-related cardiac lipotoxicity in mice.

Junyan Shi, Tak Poon, Jerry Wong, Jingchun Zhang, and Honglin Luo

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Junyan Shi

INDUCTION OF AUTOPHAGY IN COXSACKIEVIRUS B3 INFECTED MOUSE HEARTS

Background/objectives

Coxsackievirus B3 (CVB3) is one of the predominant strains of viruses causing myocarditis. CVB3 infection can attack the cardiomyocytes, which impairs heart function and may lead to dilated cardiomyophathy, a potentially lethal disorder with few treatment options. Autophagy is a cellular process by which organelles and proteins are enwrapped by double membrane vesicles, so called autophagosomes and degraded following fusion with lysosomes. Under baseline conditions autophagy represents an important homeostatic mechanism. However, the role of increased autophagy in different disease conditions is not fully understood. Increasing evidence suggests that viruses may utilize autophagy to enhance their efficiency of replication. Our previous in vitro data have shown that CVB3 infection up-regulates the formation of autophagosomes which can serve as sites for virus replication. In this study we aim to elucidate the role of autophagy in CVB3-induced myocarditis *in vivo*.

Methods

Autophagy reporter mice with GFP-labeled microtubule-associated protein 1 light chain 3 (GFP-LC3) were used to monitor autophagosome formation. Male GFP-LC3 mice at the age of 4 to 5 weeks were infected intraperitoneally with 104 pfu of CVB3 for 3 and 9 days. Mouse organs were then harvested to examine autophagosome formation by confocal microscopy and Western blotting. Furthermore, to examine the effect of inhibiting autophagy on CVB3-induced myocarditis, mice with tamoxifen-inducible cardiac-specific knockout of autophagy related gene 7 (Atg7, a gene critical for the formation of autophagosome) were generated by crossing Atg7 floxed-allele mice with MerCreMer mice under the control of -MHC promoter.

Results

Confocal microscopy analysis showed that punctuate GFP-LC3 formations were markedly increased in virus-infected heart. In line with this observation, elevated conversion of non-modified LC3-I to lipidated LC3-II was detected by Western blotting. Furthermore, we found that phosphorylation of eIF2alpha, an important regulator known to control autophagosome formation, was significantly augmented. Similar to our in vitro observation, the levels of autophagy target protein p62 appear not to be changed in virus-infected heart, suggesting that coxsackievirus infection induces autophagosome formation without enhancing protein turnover by lysosomes.

Conclusions

Our results suggest that the autophagy pathway is activated in mouse heart after viral infection as a result of the activation of upstream signaling pathway. Investigation is undergoing to examine the effect of autophagy disruption on the pathogenesis and progression of coxsackieviral myocarditis using inducible cardiac specific Atg7 knockout mice.

Farshid Garmaroudi

D Farshid S. Garmaroudi[1,2], Karin J. Holmberg[3], Jon M. Carthy[1,2], Ali Bashashati[4], Mary Zhang[1,2], Decheng Yang[1,2], Kevin A. Janes[3], David Marchant[1] and Bruce M. McManus[1,2]

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ABSTRACT #27

COUPLING BETWEEN HOST-CELL DEATH PROGRAMS BY COORDINATED ERK-P38 SIGNALING IN COXSACKIEVIRUS B3 INFECTION

Background/objectives

The host-cell response to a virus is determined in part by intracellular signaling pathways modified during infection. These pathways work together as networks in host cells, and the resulting phenotypes are interdependent, making it difficult to link virus-induced signals and particular responses at a systems level. Using coxsackievirus B3 (CVB3) infection of in vitro cardiomyocytes as a representative virus-host system, we adopted a quantitative multi-parameter approach to interrelate CVB3-induced host-cell events.

Methods

We systematically monitored the dynamics of nine signaling phospho-proteins together with six CVB3-induced host-cell readouts at five different doses of CVB3. Using Partial Least Squares, we linked CVB3-induced signaling to host-cell readouts by building a data-driven model that predicted readouts with high accuracy using two of the several assessed phospho-proteins. In further experiments, we also used small-molecule kinase inhibitors, perturbing each pathway alone or in combination.

Results

The model revealed a tight coupling between extracellular-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) signaling that had not been appreciated previously. By pharmacologically perturbing each pathway alone or in combination, we found that the ERK1/2 and ERK5 pathways redundantly enable CVB3-driven apoptosis through caspase-9 and caspase-3. Conversely, p38 signaling is required for caspase-8 activation, but remarkably, p38 plays no role CVB3-induced apoptosis. Instead, p38 appears to drive host-cell necrosis, a cell-death outcome that was embedded in our initial measurements, but is completely separable from apoptosis.

Conclusion

Thus, our systems-level analysis uncovered mechanisms, underlying two temporally coupled cell-death events, apoptosis and necrosis, that directly connect to three distinct-but-interrelated CVB3-manipulated host-cell signaling pathways: ERK1/2, ERK5, and p38.

Iran Tavakoli, Jae-Kyung Myung, Nasrin R. Mawji, Marianne D. Sadar

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Iran Tavakoli

PROTEIN-PROTEIN INTERACTIONS AS DRUG TARGETS FOR PROSTATE CANCER

Background/objectives

Castration resistant prostate cancer is suspected to be caused by over expression of androgen receptor (AR) and/or its coactivators such as CREB-binding protein and steroid receptor coactivator1-3. Our group has shown that AR can be activated via its N-terminal domain (ARN) in the absence of androgens by stimulation of the protein kinase A and Interleukin 6 (IL-6) pathways. Recently, we identified EPI-001, a small molecule that specifically blocks transactivation of the ARN by a mechanism that involves inhibiting CREB-binding protein interaction with AR and reducing AR interaction with androgen-response elements on target genes. These studies emphasize the need to identify other proteins that interact with ARN that are essential for transactivation and the protein interactions that are blocked by EPI-001.

Hypotheses: 1) Identification and inhibition of proteins that interact specifically with the ARN will block AR transcriptional activity and inhibit proliferation of prostate cancer cells.

2) EPI-001 alters the interaction of ARN with at least a subset of the identified interacting proteins.

Methods

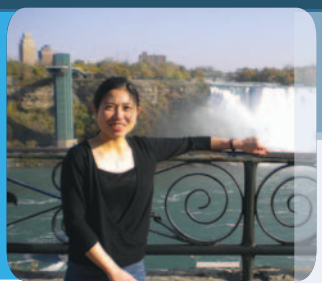
We created an expression vector for a chimeric protein of the ARN with 3x FLAG (FLAG-ARN). FLAG-ARN was transfected into LNCaP human prostate cancer cells that were then treated with 50 ng/ml IL-6 (simulates ARN activation). ARN and the interacting proteins were co-immunoprecipitated (Co-IP) using ANTI-FLAG M2 Affinity gel. Vector construct with no ARN was used as a negative control. Proteins were separated using a 1 dimensional PolyAcrylamide Gel Electrophoresis and stained using Colloidal Coomassie or Silver staining kits. Gel bands differentially expressed between vector and ARN transfected samples were excised and analyzed by electrospray-tandem mass spectros.

Results

Co-IP coupled to mass spectrometry analysis identified 25 novel proteins that interact with ARN. Protein 1 (P1), a 37 kDa protein involved in cell attachment and migration, was selected as a candidate for further analysis. We confirmed interaction of ARN with P1 by Co-IP-western blot analysis. We also showed that FLAG-ARN does not interact with the endogenous full length AR in LNCaP cells.

Conclusions

In this study, we identified proteins that specifically interact with the ARN. Discovery of these interactions, and changes in response to EPI-001, will yield insight into the mechanism of transactivation of the ARN to develop new drugs to delay or cure castration resistant prostate cancer.



Yu-Hsuan Huang, Brian K. Chung, John J. Priatel and Rusung Tan

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

Yu-Hsuan (Lynn) Huang

SAP POSITIVELY REGULATES THE DIFFERENTIATION OF IL-17 PRODUCING CD4 AND CD8 T CELLS

Background/objectives

X-linked lymphoproliferative disease (XLP) is a severe immunodeficiency caused by mutations in SLAM-associated protein (SAP) and humans with XLP often develop fatal complications upon exposure to Epstein-Barr virus (EBV). Previous studies demonstrate that SAP regulates the differentiation of CD4 helper T (Th) type 2 cells, however, it is unclear how this deficit could lead to loss of viral control. Here, we have examined the role of SAP in regulating the differentiation of the recently identified subsets of IL-17 secreting CD4 (Th17) and CD8 (Tc17) T cells which involve in regulating viral persistence.

Methods

First, proportions of Th17 and Tc17 cells from wild-type and SAP-deficient (SAP-/-) mice were compared under in vitro polarizing condition. Subsequently, to study the role of SAP in regulating Th17 and Tc17 cell differentiation in vivo, we induced experimental autoimmune encephalomyelitis (EAE) in wild-type and SAP-/- mice.

Results

In vitro experiments revealed that T cells from SAP-/- mice exhibit a greatly diminished potential to differentiate into Th17 and Tc17 cells as compared to wild-type T cells under IL-17 polarizing conditions. In vivo experiments of EAE induction, SAP-/- mice displayed reduced disease progression, greatly decreased numbers of CNS-infiltrating T cells and diminished frequencies of resident IL-17 secreting T cells relative to wild-type animals.

Conclusion

Together, our results suggest that SAP positively regulates the differentiation of naïve T cells into IL-17 producing effectors. Recent work has shown that IL-17 plays critical roles in host defense and, together with our findings, now suggests that IL-17 may be critical for mounting anti-viral immunity against EBV.

Alicia A. Tone[1-2], Carl Virtanen[3], Patricia Shaw[2,4] and Theodore J. Brown[2]

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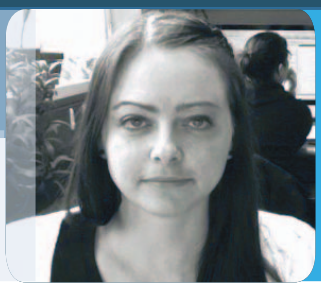


Alicia Tone

ALTERED PRO-INFLAMMATORY GENE EXPRESSION IN BRCA1-MUTATED LUTEAL PHASE FALLOPIAN TUBE EPITHELIUM: EVIDENCE FOR A DIRECT ROLE OF BRCA1 AND DAB2 IN MEDIATING ANTI- VS. PRO-INFLAMMATORY SIGNALLING

Background/objectives

We previously obtained and compared gene expression profiles from laser-capture microdissected non-malignant fallopian tube epithelium (FTE) from BRCA1/2-mutation carriers (FTEb) and control patients, and adnexal high-grade serous carcinoma (HGSC) to identify alterations predisposing to malignant transformation. Notably, FTEb samples obtained during the luteal phase showed global gene expression profiles closely resembling HGSC specimens and exhibited decreased expression of the adaptor molecule disabled homolog 2 (DAB2). Initial gene expression analysis revealed that 110/630 (17.5%) of differentially expressed genes with known function are involved in inflammation or immune response, consistent with ovulation being an acute inflammatory event. The objective of this study was to perform a comprehensive analysis of inflammatory gene expression in these samples, and to determine whether BRCA1 or DAB2 are directly involved in regulation of nuclear factor-kB (NFkB)-mediated pro-inflammatory signalling.

Methods

To characterize inflammatory gene expression in profiled FTE and HGSC samples, a list of NFkB-dependent pro-inflammatory genes was compiled through a combination of gene set enrichment and PubMed database search. Corresponding probesets for all genes were identified, filtered according to level of expression, and unpaired t-tests were performed to identify differentially expressed genes. To determine the impact of DAB2 and BRCA1 on NFκB activity, cytokine-responsive ES2 and A549 cells were co-transfected with DAB2- or BRCA1-specific siRNA and an NFkB-responsive luciferase reporter, treated with 10ng/mL of the ovulation-associated cytokine TNFa or vehicle, and harvested 8h later for luciferase activity determination. To determine if DAB2 or BRCA1 alters mRNA expression of NFkB target genes, cells were transfected with DAB2 or BRCA1 siRNA, treated with TNFa and harvested for total RNA extraction and RT-qPCR.

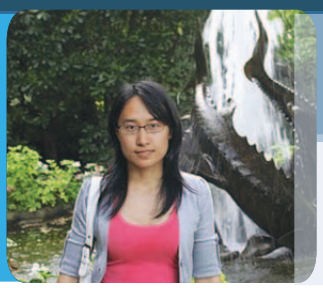
Results

124 inflammation-associated gene probesets were differentially expressed in a subset of BRCA1-mutated luteal phase samples we previously found to group with HGSC (referred to as FTEb(S)), relative to remaining FTE samples. FTEb(S) samples also differentially expressed 264 inflammation-associated probesets relative to the remaining luteal phase samples likely exposed to the same post-ovulatory environment. Both BRCA1- and DAB2-specific siRNA increased TNFa-induced NFκB activity relative to non-targeting siRNA, suggesting that both proteins may directly repress pro-inflammatory signalling. Consistently, DAB2 siRNA combined with TNFa treatment greatly induced mRNA expression of the NFkB-dependent target gene SOD2.

Conclusions

These data provide evidence of elevated pro-inflammatory signalling in a subset of BRCA1-mutated luteal phase FTE, consistent with an altered response to ovulation-associated cytokines that are locally elevated during this phase. Furthermore, we demonstrate direct effects of both BRCA1 and DAB2 on NFkB activity, suggesting a novel link between BRCA mutation status, ovulation and predisposition to HGSC.

ABSTRACT #30



Qianli Ma[1,2], Adrian Wan[1,2], Gregg Morin[1,2], Catherin Pallen[1], Sohrab Shah[1], Samuel Aparicio[1,2]

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Qianli Ma

STUDY OF MOLECULAR FUNCTION AND SUBSTRATE SPECIFICTY OF PROTEIN PHOSPHOTASE 2A REGULATORY SUBUNIT PPP2R2A

Background/objectives

Phosphorylation and dephosphorylation is a tightly controlled form of signaling pathways involved in cell growth and division. Therapies targeting kinase phosphorylation has been an effective way of cancer treatment. Phosphotases controls dephosphorylation of smaller sets of targets, however, are relatively under-explored. Protein phosphatase 2A (PP2A) is a type of serine/threonine phosphatase, which consists 1% of cellular proteins. It is a tri-subunit complex containing a scaffold subunit, catalytic subunit and regulatory subunit. PP2A has been found to play a significant role in tumor suppression. Deletion and inhibition of PP2A subunits have been identified in various cancers. PP2A regulatory subunit is believed to determine the specificity of the PP2A function. SV40 has also been found to transform cells by replacing regulatory subunits to inhibit the function of the PP2A complex. In our previous data, we identified PPP2R2A, a PP2A B family regulatory subunit, is frequently deleted in breast tumors, especially in luminal B subtype. We hypothesize that PPP2R2A plays a critical role in cell cycle progression and determines specific pathways of PP2A complex. The objective of this study is to determine functional consequences of PPP2R2A subunits copy number loss and its downstream substrate specificity in breast cancer model.

Methods

We will generate PPP2R2A depletion cells using siRNA/shRNA knockdown and PP2A deficient cell model by inactivating PP2A with okadaic acid, a PP2A specific inhibitor. We will assay cell proliferation, cell cycle progression and apoptosis in both systems and identify the aspects of cellular functions that PPP2R2A specifically involved. We will also study cell transformation of PPP2R2A depleted cells using soft agar colony formation assay. Moreover, we will develop isogenic cell lines using homologous recombination mediated knockout and further study PPP2R2A substrate specificity using stable isotope labeling with amino acids in cell culture (SILAC) quantitative proteomic profiling method or phospho-site mass spectrometry antibody screen.

Results

Preliminary results have shown that PPP2R2A is a important regulator in cell cycle mitotic exit. PPP2R2A depletions in cells significantly delays mitosis.

Conclusion

PPP2R2A is a specific regulatory subunit of PP2A complex controlling cell cycle progression. Our further study will focus on studying the downstream substrate specificity of PPP2R2A to elucidate the signaling pathway it is involved. The results would provide potential targets for future therapeutic drug development.

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Qianli Ma

HIGH CONTENT IMAGING SCREENING OF CELL CYCLE AND CELL PROLIFERATION ANALYSIS FOR IDENTIFYING CANCER DRIVER GENES

Background/objectives

Somatic copy number aberrations is a dominant feature of cancer genome. Our group previous conducted a genome copy number and transcriptome analysis of 997 breast tumor samples. The study identified 1100 copy number amplified genes and 66 copy number deleted genes with correlated changes in gene expression level. The copy number changes of these genes define the landscape of genomic aberrations of breast cancer, but comprehensive functional analysis of these genes copy number changes remains unexplored. We hypothesize that high content imaging analysis of cell cycle and proliferation is able to identify critical genomic aberrant genes involved in cell transformation.

Objective: To identify cancer driver genes by combined cell cycle analysis with high content imaging screening and cell proliferation analysis with fluorescent intensity detection.

Methods

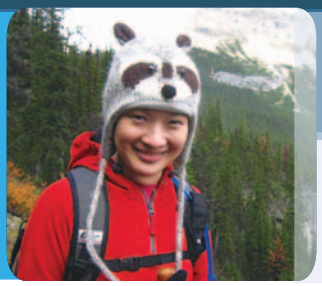
We will use high content imaging approach to screening a panel of breast cancer cell lines for cell cycle analysis. Cell lines will be chosen based on Affymetrix SNP array copy number analysis. The panel of cell lines will cover the list of genes with copy number amplification and deletions identified from genomic landscape study. We will use siRNA to knockdown genes with copy number gain and introducing cDNA of gene with copy number loss. We will conduct cell cycle analysis by labeling DNA content with DAPI, labeling S phase cells with BrdU , and mitotic cells with phospho-H3. The screen will be carried out in microplates and images will be captured on INCell Analyzer 1000. Cell proliferation will be labeled with Ki67 and apoptotic cells will be labeled with Caspase3. The fluorescent intensity will be detected on Tecan Safire microplate reader. Gene with obvious cell cycle aberrations, cell growth arrest or increased apoptosis level will be identified from the combined analysis for further study.

Results

Preliminary results shows that the high content imaging of BrdU and phospoh-H3 label cells is able track changes of cellular progression. The fluorescent intensity is able to detect changes in cell proliferation and apoptosis.

Conclusions

The combined high content fluorescent analysis with high content imaging and fluorescent intensity analysis is a comprehensive method of identifying copy number aberrant genes involved in cell malignant transformation.



Lisa Ang

Lisa S. Ang[1], Wendy A. Boivin[1], Sarah J. Williams[1], Hongyan Zhao[1], Bruce M. McManus[1], Michael F. Allard[1], R. Chris Bleackley[2], David J. Granville[1]

[1]James Hogg Research Centre, Providence Heart + Lung Institute, Department of Pathology and Laboratory Medicine, University of British Columbia; [2]Department of Biochemistry, University of Alberta

INHIBITION OF EXTRACELLULAR GRANZYME B REDUCES ABDOMINAL AORTIC ANEURYSM RUPTURE

Background/objectives

Abdominal aortic aneurysm (AAA) is an age-related disease caused by progressive weakening of the vessel wall. Although AAA progression leading to rupture can be fatal, effective pharmacological interventions aimed at halting AAA progression at early stages of disease are not available. Previous work in our laboratory has demonstrated that knocking out the serine protease granzyme B (GZMB) reduces incidence and severity of AAA in mice in a perforin-independent manner. GZMB is well known for its role in eliminating target cells via apoptosis, but also accumulates extracellularly during inflammation and cleaves extracellular matrix (ECM) components such as fibronectin and fibrillin-1. We hypothesize that GZMB contributes to AAA development via the degradation of vascular extracellular matrix components and that the inhibition of extracellular GZMB will reduce incidence and severity of AAA progression.

Methods

To induce aortic aneurysm, apoE-/- mice were implanted with an osmotic minipump that released angiotensin II (1000 ng/min/kg) for 28 days. Animals were injected with a novel GZMB inhibitor, serpin A3N, (4-120 ug/kg) or saline prior to pump implantation. Tissues were harvested after 28 days. Survival, morphology and ECM composition were evaluated.

Results

A significant dose-dependent reduction in the frequency of aortic rupture was observed in mice that received serpin treatment. Serpin A3N was also shown to prevent decorin cleavage by GZMB in vitro. Immunohistological analyses revealed reduced GZMB staining and a corresponding reduction in loss of adventitial decorin in serpin-treated mice while collagen deposition was increased. When compared to controls, adventitial collagen from serpin-treated mice was observed to have significantly thicker fibrils and greater packing density when evaluated by second harmonic generation, suggesting higher tensile strength.

Conclusion

GZMB contributes to the loss of vessel wall integrity in AAA through the cleavage of ECM components. Extracellular inhibition of GZMB prevents degradation of decorin and promotes beneficial remodelling of collagen. This reinforcement of the adventitia following medial injury reduces the rate of rupture and improves the overall rate of survival.

Wendy A. Boivin[1,2], Lisa S. Ang[1,2], Marlo Shackleford[1], Hongyan Zhao[1], Tillie L. Hackett[1,3], Darryl A. Knight[1,3] and David J. Granville[1,2]

[1]UBC James Hogg Research Centre, St. Paul's Hospital; [2]Department of Pathology and Laboratory Medicine, Faculty of Medicine, UBC; [3]Department of Anesthesiology, Pharmacology and Therapeutics, UBC

Wendy Boivin

GRANZYME B CLEAVES PROTEOGLYCANS AND RELEASES SEQUESTERED TGF- (BETA) FROM EXTRACELLULAR MATRIX

Background/objectives

Granzyme B (GrB) is a cytotoxic serine protease that is elevated in chronic inflammation and disease and contributes to tissue damage though immune-mediated apoptosis and the degradation of extracellular matrix (ECM). During inflammation, GrB accumulates in the extracellular milieu, retains its activity and has been implicated in atherosclerosis and abdominal aortic aneurysm, yet the mechanisms behind its extracellular activity remain largely unknown. We hypothesize that GrB cleaves proteoglycan substrates, having implications in transforming growth factor-beta (TGF-β (beta)) bioavailability.

Methods

For ECM cleavage assays, GrB and the inhibitor 3,4-dichloroisocoumarin (DCI), were incubated for 4 and 24 h at room temperature, with decorin, biglycan or soluble betaglycan and visualized by Ponceau staining. Cleavage of biglycan and decorin was confirmed with proteolysis assays on human coronary artery smooth muscle cell (HCASMC)-derived ECM. Cleavage fragments were subjected to Edman degradation for cleavage site identification. As TGF-beta is sequestered by the aforementioned proteoglycans, GrB was incubated with TGF-beta1 bound proteoglycans or SMC-derived ECM to determine if GrB cleavage resulted in the release of sequestered TGF-beta. Cytokine release was assessed in supernatants using Western blotting. To determine if the TGF-κ released by GrB was active, supernatants from the above release assay were incubated on HCASMC and SMAD activation was examined by Western blotting.

Results

GrB cleaved decorin, biglycan and betaglycan, with proteolysis evident at GrB concentrations as low as 25 nM. Proteolysis was inhibited by DCI but not the solvent control DMSO, and decorin and biglycan cleavage was also evident in HCASMC-derived ECM. Edman degradation analysis determined GrB cleavage sites in the proteoglycans with P1 residues of aspartic acid, consistent with GrB cleavage specificity. In cytokine release assays, TGF-beta was liberated GrB-dependently from decorin, biglycan, and betaglycan, as well as from SMC-derived ECM, after 24 h of incubation. TGF-beta was not released in the absence of GrB or when GrB was inhibited by DCI, indicating release from decorin, biglycan and betaglycan was specific. In addition, the TGF-beta liberated by GrB remained active and induced SMAD-3 phosphorylation in HCASMC, after 20 min of incubation.

Conclusions

In addition to contributing to ECM degradation and influencing tissue structural integrity in vivo, extracellular GrB activity also induces the release of active TGF-beta from proteoglycans, with potential implications in chronic inflammatory disease.

Eugene Chu

Eugene M Chu[1], Daven C Tai[1], Jennifer L Beer[1], John S Hill[1]

[1]UBC James Hogg Research Centre, Heart and Lung Institute, St. Paul's Hospital and [2]Department of Pathology and Laboratory Medicine, University of British Columbia, BC, Canada

MACROPHAGE HETEROGENEITY IS ASSOCIATED WITH DIFFERENCES IN CHOLESTEROL METABOLISM

Background/objectives

Macrophages are heterogeneous in nature and may play different roles in atherosclerosis progression. Previous studies have shown that macrophages can vary in their function depending on the specific nature of the cytokine environment. Our objective was to evaluate the ability of different monocyte-derived macrophage (MDM) sub-phenotypes to maintain cholesterol homeostasis.

Methods

Primary human monocytes were differentiated into macrophages and subsequently treated with cytokines including interleukin-4 and interleukin-13 (IL4/13), interferon gamma and tumor necrosis factor alpha (IFNg/TNFa), or interleukin-10 to induce unique functional phenotypes. Oxidized LDL (oxLDL) cellular association, cholesterol accumulation, and apolipoprotein A-I (ApoA-I)-mediated cholesterol efflux was evaluated and compared to untreated controls. Expression of targets likely to be involved in oxLDL metabolism such as CD36, SR-AI, PPARgamma (PPARg) and ABCA1 were measured.

Results

IFNg/TNFa treated MDMs showed an 86% reduction of oxLDL cellular association ($p<0.05$), a 15% decrease in cholesterol accumulation ($p<0.01$), and a 55% reduction in apoA-I-dependent cholesterol efflux. IFNg/TNFa treatment was also associated with a 49% reduction in CD36 mRNA and protein as assessed by flow cytometry ($p<0.01$, $p<0.05$), a 62% decrease in PPARg mRNA, a 24% reduction in ABCA1 mRNA but little change in SR-AI mRNA levels. IL4/13 treatment was associated with a 2.5-fold increase in cholesterol efflux ($p<0.01$), a 25% reduction in oxLDL cellular association, and little change in cholesterol accumulation. IL4/13 treatment was also associated with a 1.3-fold increase in ABCA1 mRNA, a 49% decrease in CD36 mRNA ($p<0.001$) and 67% decrease in CD36 protein ($p<0.01$), a 2.8-fold increase in PPARg ($p<0.01$) but little change in SR-AI mRNA levels. The effects of the IL10 treatment did not differ greatly from the untreated control for all of the functional assays.

Conclusion

Macrophages treated with either IFNg/TNFa or IL4/13 have distinct changes in cholesterol metabolism associated with cholesterol efflux and oxLDL cellular association compared to untreated cells. Some of these differences are likely related to observed changes in the expression of ABCA1, CD36, and PPARg.

Daven C. Tai[1], Jennifer L. Beer[1], Eugene M. Chu[1], Kenneth W. Harder[2], John S. Hill[1]

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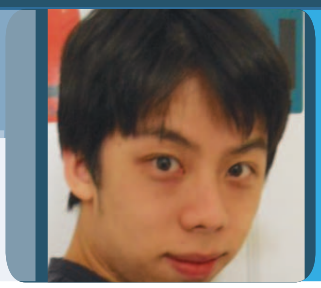

Daven Tai

INVESTIGATION OF MACROPHAGE HETEROGENEITY IN ATHEROSCLEROSIS

Background/objectives

Macrophages are key regulators of inflammation and cholesterol metabolism in atherosclerosis. Depending on the cytokines in their environment, macrophages can exhibit differing activation states. Interferon (IFN)-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. It has been proposed that classically activated macrophages are pro-inflammatory and therefore more atherogenic than alternatively activated macrophages. However, detailed characterization of the atherogenicity of polarized macrophages in vitro, as well as the effect of modulating macrophage phenotype in vivo on atherosclerosis, remains to be elucidated. Our goal is to determine the atherogenic potential of different macrophage activation states and whether the absence of STAT6 on macrophages will influence atherosclerosis development in mice.

Methods

Reactive oxygen species (ROS) production and cholesterol metabolism were measured as an indication of macrophage atherogenicity. Bone marrow cells from C57Bl/6 mice were differentiated into macrophages in macrophage colony stimulating factor. Macrophages were treated with 10 ng/mL of IL4 or IFN-gamma for 24 hours and then loaded with 50 ug/mL of copper-oxidized LDL (oxLDL) for 24 hours. Cellular ROS production was measured using the CM-H2DCFDA reagent while mitochondria-specific superoxide was measured using the MitoSOX reagent (Invitrogen).

The capability of bone marrow-derived macrophages from wild type (WT) and Stat6-/- C57Bl/6 mice to efflux cholesterol to apolipoprotein-AI (ApoA-I) or high density lipoprotein (HDL) was measured by a radiolabeled cholesterol efflux assay. The rate of oxLDL cellular association was measured using DiI-labeled oxLDL. The ability of the cells to retain cholesterol was measured via an oxLDL accumulation assay. STAT6 deficient bone marrow was also transplanted into lethally irradiated LDL receptor null mice for atherosclerosis studies.

Results

Our preliminary data shows increased cellular ROS production in IL4-treated compared to untreated murine macrophages. In addition, mitochondria-specific superoxide production in IFN-gamma and IL4-treated macrophages was 88% and 140% of untreated control, respectively. Cholesterol efflux to ApoA-I and HDL, cellular association of DiI-oxLDL, and cholesterol accumulation were not significantly different between WT and Stat6-/- macrophages. The analysis of atherosclerosis lesion formation in mice transplanted with STAT6 deficient bone marrow is currently ongoing.

Conclusions

Alternatively activated murine macrophages appear to have increased ROS production compared to untreated and classically activated macrophages. In addition, the absence of STAT6 alone in normal culture conditions did not alter macrophage cholesterol metabolism.

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[1]British Columbia (BC) Cancer Agency; [2]University of British Columbia

Kimberly C. Wiegand

ARID1A MUTATIONS IN ENDOMETRIOSIS-ASSOCIATED OVARIAN CARCINOMAS

Background/objectives

Ovarian clear cell (CCC) carcinomas often arise from endometriosis, however the genetic events associated with this transformation are unknown. CCCs are the second leading cause of ovarian cancer deaths, and respond poorly to standard treatment. Using a next generation sequencing approach, we attempted to clarify the molecular events involved in transformation of endometriosis into this understudied subtype of ovarian carcinoma.

Methods

We sequenced the whole transcriptomes of 18 clear cell carcinomas and a CCC cell line and found truncating mutations in ARID1A in 6 cases. We then sequenced ARID1A in an additional 210 ovarian carcinomas and a second CCC cell line and measured BAF250a expression by immunohistochemisty (IHC) in 455 additional ovarian carcinomas. In two CCCs with contiguous atypical endometriotic epithelium, we sequenced DNA microdissected from the atypical endometriotic epithelium to determine whether ARID1A mutations are early events.

Results

Truncating and somatic missense mutations, and one ARID1A rearrangement were seen in 55/119 (46%) of clear cell carcinomas, 10/33 (30%) endometrioid carcinomas (EC), and 0/76 high grade serous (HGS) ovarian carcinomas. All truncating mutations for which germline DNA was analyzed were somatic. The correlation between ARID1A mutations and BAF250a expression was evaluated by IHC staining for BAF250a in 73 CCCs, 33 ECs and 76 HGS carcinomas from both the discovery and mutation validation cohorts. The presence of truncating mutations in ARID1A was significantly associated with BAF250a loss in endometriosis associated cancers (Fisher Exact $p<0.001$). Within CCCs, and ECs, 27/37 (73%) and 5/10 (50%) cases with mutations showed loss compared to 4/36 (11%) and 2/23 (9%) of mutation negative cases, respectively. Loss of BAF250a expression is strongly associated with endometriosis-related ovarian cancers (31/73 (42%) of CCCs and 7/33 (21%) of ECs) compared to high grade serous cancers (1/76 (1%)) (Fisher Exact $p<0.001$). Two CCCs with BAF250a expression loss and ARID1A mutations had atypical (contiguous) endometriosis available; both mutations were present in the tumor and the contiguous atypical endometriosis but not in a distant endometriotic lesion.

Conclusion

These data implicate ARID1A as a tumor suppressor gene frequently disrupted in CCCs and ECs. As ARID1A mutation and loss of BAF250a can be seen in the pre-neoplastic lesions, this is an early event and likely critical in the transformation of endometriosis into cancer.

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Jasmine Hamilton

NOVEL MACROMOLECULAR IRON CHELATORS: AN INNOVATIVE APPROACH TO THE TREATMENT OF TRANSFUSION ASSOCIATED IRON OVERLOAD

Background/objectives

Inherited hemoglobin disorders are the most common single gene disorders in man; affecting more than 330,000 births annually. Patients are treated with transfusion therapy (TT) which ameliorates severe anemia and prolongs survival. However, since the human body cannot actively excrete iron, TT causes body iron to invariably increase to toxic levels. Excess iron causes damage to major organs such as the liver, heart, and pancreas. Iron chelators are used clinically for the treatment of iron overload but are expensive, and have significant side effects which limit patient use. Deferoxamine (DFO), the most widely used iron chelator, must be continuously injected under the skin, for 8-12 hours per day, at least five times per week in order to have the desired effect. Moreover, other chelating drugs are ineffective at promoting adequate iron excretion. Thus, the development of iron chelators that are less toxic, and more selective than current chelators will reduce the systemic toxicity of current iron chelators while optimizing their therapeutic efficiency.

Methods

Hyperbranched polyglycerols (HPGs) with molecular weights (MW) ranging from 50-500 kDa were conjugated to various numbers of DFO molecules. The iron-binding efficiency of novel conjugates (HPG-DFOs) was determined by analyzing the UV-Visible Spectra of HPG-DFOs after complexation with iron. UV-Visible Spectroscopy of hemoglobin (HbA) was investigated to determine whether HPG-DFOs prevent iron mediated oxidation of proteins in a similar manner as DFO. Blood compatibility of HPG-DFOs was analyzed by coagulation assays including Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and Thromboelastography (TEG); platelet activation, and red cell aggregation analyses. The toxicity profiles of HPG-DFOs were tested in vitro using Human Umbilical Vein Endothelial cells (HUVECs). In vivo tolerance was determined by measuring the serum lactate dehyrdogenase (LDH) levels and body weight of Balb/cJ mice over a 28-day period after subcutaneous injections.

Results

HPG-DFOs incubated with iron produced a lambda max of 429 nm, indicating that the iron binding properties of DFO remained unaffected after conjugation to HPGs. Conjugates were also effective at preventing iron-mediated oxidation of hemoglobin in our HbA model. In vitro blood compatibility analysis did not show any significant changes in blood coagulation, platelet activation, hemolysis, or red cell aggregation after conjugation. HUVEC based cell viability assays showed that the cytotoxicity of HPG-DFOs decreased more than 100-fold at identical concentrations of DFO. Most importantly, serum LDH levels of Balb/cJ mice injected with HPG-DFOs did not differ from that in control mice, and there was no visible weight loss indicative of toxicity.

Conclusions

The HPG-DFO conjugates described are considerably less toxic to mammalian cells than DFO and were able to inhibit Fe (III)-mediated oxidation of proteins, the underlying pathology of Sickle Cell Anemia and Beta Thalassemia. These novel chelators are also expected to reduce the iron mediated toxicity associated with repeated blood transfusions, and are thus promising candidates for further development.

Manu Thomas Kalathottukaren[1], Rajesh.A.Shenoi[1], Benjamin F.L.Lai[1], Dirk Lange[3], Donald.E.Brooks[1,2], Ben Chew[3], Cedric Carter[1] and Jayachandran. N. Kizhakkedathu[1,2]

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Manu Thomas Kalathottukaren

A NOVEL POLYMER BASED ANTAGONIST FOR CLINICALLY USED HEPARIN ANTICOAGULANTS

Background/objectives

Anticoagulants are used for the treatment and prevention of thromboembolic disorders such as venous thromboembolism, which is one of the leading causes of mortality and morbidity around the globe. Unfractionated heparin (UFH) has been widely used for anticoagulation therapy particularly in cardiovascular surgeries. However, UFH is associated with risk of severe bleeding and heparin- induced thrombocytopenia, which is an immune mediated prothrombotic disorder. The only clinically approved antidote to UFH is protamine which is a cationic peptide containing approximately 67% of arginine. However, protamine induces severe side effects ranging from mild hypertension to idiosyncratic fatal cardiac arrest. In addition, protamine neutralizes LMWHs only partially and is ineffective against the synthetic pentasaccharides such as fondaparinux. So, there is an urgent need for a safer and efficient universal antidote which could neutralize all the available anticoagulants. Here, we report the synthesis of novel hyperbranched polyglycerol- based cationic polymer and its ability to neutralize UFH and LMWHs in vitro and *in vivo*.

Methods

Hyperbranched polyglycerol was synthesized by anionic ring opening polymerization of glycidol using trimethylolpropane/potassium methylate as initiator. Subsequently MPEG epoxide 400 was added drop wise over a period of 12hrs.The polymer was dissolved in methanol and precipitated from diethyl ether. A portion of the hydroxyl group of the polymer was converted in to tosylate by reaction with p-toluenesulfonyl chloride in pyridine, followed by refluxing with tris(2-aminoethylamine) in 1,4-dioxane for 24 hrs to generate the amine-functionalized polymer. The polymer was finally reacted with formaldehyde/formic acid in water to afford the cationic HPG-PEG-Amine. The polymer was characterized by gel permeation chromatography (GPC) and NMR analysis. Blood compatibility of the polymer was evaluated using TEG, APTT and PT assays. Neutralization of UFH and LMWHs by the new polymer was studied in vitro and in vivo. Toxicity of the new polymers was determined by injecting escalating doses in mice.

Results

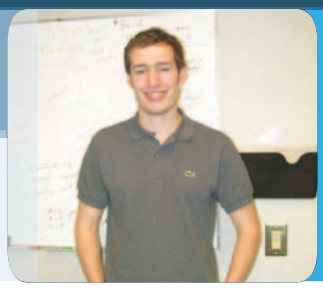
The cationic HPG-PEG-Amine neutralized both UFH and LMWHs over a broad range of concentration (0.025 -0.25 mg/ml) without any side effects both in vitro and in vivo. On the other hand, protamine neutralized the heparins only in the narrow concentration range of 0.025-0.05mg/ml, at higher concentrations protamine itself acts as an anticoagulant and did not neutralize LMWHs. The polymer showed excellent blood compatibility and cell compatibility compared to protamine. Dose tolerance studies in mice revealed that polymer is non-toxic up to 200mg/kg, which is 10 fold higher than the maximum tolerated dose of protamine.

Conclusion

We have developed a novel, biocompatible and non-toxic polymer based antidote which exhibit complete reversal of anticoagulant effects of UFH and LMWHs both in vitro and in vivo. Development of polymeric antidote for the synthetic pentasaccharides based anticoagulants such as fondaparinux is in progress.

Greg Stewart, Jennifer Y. Kennett, Daiana Becker, Kelsie L. Thu, Emily A. Vucic, Larissa A. Pikor, Raj Chari, Wan Lam, Stephen Lam

British Columbia Cancer Research Centre, Vancouver, Canada



Greg Stewart

GENDER DISPARITIES IN LUNG ADENOCARCINOMA GENOMES

Background/objectives

Gender disparities related to incidence, clinical presentation, mortality and therapeutic response in non small cell lung cancer (NSCLC) are becoming increasingly apparent. It is unclear whether environmental, behavioral, hormonal, genetic, or biological factors, including nicotine clearance, can fully explain such disparities. For example, women on hormone replacement therapy have an increased risk of NSCLC, but these changes cannot explain observed clinical disparities. Recent findings in never smokers with adenocarcinoma, have shown females display a higher rate of EGFR mutation than males. It is possible that other gender based genetic differences play a role in susceptibility to lung cancer in smokers. We hypothesize that lung tumor genomes of males and females smokers harbor differences at the molecular level that may underlie the gender based disparities observed in adenocarcinoma.

Methods

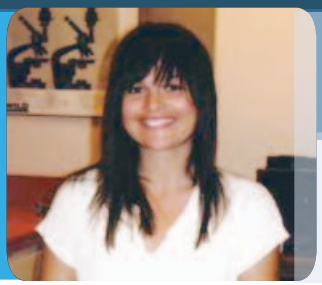
Global gene dosage profiles for 48 lung adenocarcinoma from 16 males and 32 females (all current smokers) were generated by array comparative genomic hybridization. A Fisher's test was used to identify genomic regions differentially altered between males and females. The identified candidate regions from our discovery set were validated in an external cohort comprised of male (n=29) and female (n=37) current smokers with lung adenocarcinoma from the database of Genotypes and Phenotypes (dbGaP).

Results

We found 7 distinct differentially altered regions between male and female lung adenocarcinoma, that overlapped between our discovery and external datasets, which encompassed 200 genes. Using Ingenuity Pathways Analysis we identified several pathways and biological functions affected by genes located in these regions. Many of these genes are known oncogenes previously shown to be involved in NSCLC and inflammatory pathways such as PI3/AKT and IL6, while others are new. These findings imply that these differentially altered regions and the genes they encompass may play a significant role in gender differences observed in NSCLC.

Conclusions

Our study suggests there are gender specific genetic disparities in NSCLC at the gene dosage level, and provides rationale for further investigation in larger cohorts. On-going studies that include analyses of different genomic dimensions such as gene expression and DNA methylation, may lead to additional information on gender differences in NSCLC, potentially leading to improvements in prevention, diagnosis, and treatment.



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Emily Vucic

THE ROLE OF DNA METHYLATION IN DEVELOPMENT OF LUNG CANCER IN SMOKERS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Background/objectives

Patients with chronic obstructive pulmonary disease (COPD) have an increased risk of developing lung cancer. Our overall goal is to identify molecular pathways associated with the link between COPD and lung cancer. We hypothesize that COPD related DNA methylation changes constitute early events in tumour suppressor gene inactivation, and in order to inactivate both alleles, a second gene dosage alteration in tumours may be found at these genes. Furthermore, we hypothesize that genes uniquely inactivated in tissues from COPD patients with non small cell lung cancer (NSCLC), compared to similar tissues from patients with NSCLC alone, can be used to identify molecular pathways causal to lung tumorigenesis in COPD patients.

Methods

We generated DNA methylation and copy number profiles for NSCLC tumour and matched non-malignant lung tissues from smokers with (n=10) and without (n=15) COPD. Methylation profiles were also generated for bronchial epithelial cells from small airways of smokers with COPD alone (n=24), and COPD and NSCLC (n=15). Genes that were 1) differentially methylated between non-malignant lung tissue from COPD patients with NSCLC compared to similar tissue from patients with NSCLC alone, 2) additionally altered at the level of gene dosage in COPD tumours compared to tumours from non COPD patients, and 3) differentially methylated in bronchial epithelial cells from COPD patients with NSCLC compared to COPD alone, were selected for gene function and pathway analysis.

Results

We identified 544 genes that were hypermethylated in non-malignant lung parenchyma tissues and concordantly lost at the DNA level in corresponding tumours from COPD patients, but not in similar tissues from non COPD patients. Of these genes, 174 were also hypermethylated in airway cells of patients with COPD and NSCLC compared to those with COPD alone. These COPD+NSCLC specific genes corresponded to the PI3K/AKT, aryl hydrocarbon signalling and retinoic acid mediated apoptotic pathways.

Conclusions

Our results suggest NSCLC in COPD patients may develop through unique tumour promoting and metabolic pathways. Understanding COPD specific NSCLC molecular biology may lead to development of targeted chemoprevention strategies specific to the NSCLC promoting aspects of COPD.

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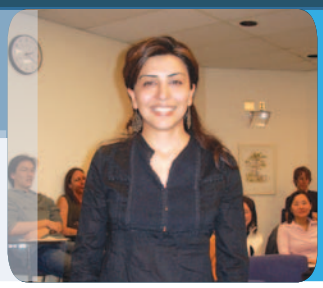
[1]UBC James Hogg Research Centre, Department of Pathology, [2]Canada's Michael Smith Genome Sciences Centre



Seti Boroomand

TRANSDIFFERETIATION OF VALVULAR INTERSTITIAL CELLS TO AN OSTEOBLAST-LIKE PHENOTYPE; AN IN VITRO MODEL FOR CALCIFICATION

Background/objectives

Hypercholesterolemia, a significant cardiovascular (CV) risk factor, induces calcification [mineralization] of aortic valves through the induction of valvular myofibroblast differentiation into an osteoblast-like phenotype, leading to a condition known as Aortic Stenosis (AS). A significant player in the pathway involved in this process, is the wnt/beta-catenin signaling pathway co-receptor, low density lipoprotein (LDL) receptor-related protein 5 (LRP5), which is an important regulator of osteoblast cell differentiation in normal skeletal bone formation. The pathologic form of calcification results in the expression of alkaline phosphatase, osteonectin, osteocalcin and the bone specific transcription factor Cbfa1 in cells. Valvular interstitial cells (VICs) undergoing osteoblastic transformation contributes to the osteogenic program of vascular calcification which clearly resembles the process of skeletal bone ossification. Bone morphogenic proteins (BMPs) and wnt ligands have been implicated in the pathologic calcification and the osteoblastic transdifferentiation of aortic VICs. In this study we examined the process of transdifferentiation of human VICs to an osteoblast-like phenotype.

Objective: Wnt/beta-catenin signaling induces pro-osteogenic gene program pathways in VICs, resulting in the promotion of calcification, a known cardiovascular risk factor which can promote calcification that then may enhance cardiovascular risk factors. We aim to determine whether wnt/beta-catenin signaling is critical in the activation of the pathological mineralization process. Also verify that the wnt/beta-catenin inhibition can reduce and/or reverse the calcification process in VIC.

Methods

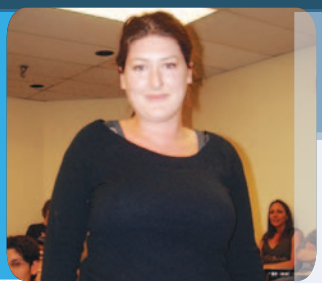
Human VICs have been isolated from transplanted heart patients without ischemic heart disease and cultured in osteogenic condition medium before they were collected at different specific time points over the course of 4 weeks. Cells were fixed and stained for calcium and alkaline phosphatase as indicators of calcification. Alkaline phosphatase activities were also measured for confirmation. Protein and expression studies are being performed to look at other osteogenic markers.

Results

We identified 544 genes that were hypermethylated in non-malignant lung parenchyma tissues and concordantly lost at the DNA level in corresponding tumours from COPD patients, but not in similar tissues from non COPD patients. Of these genes, 174 were also hypermethylated in airway cells of patients with COPD and NSCLC compared to those with COPD alone. These COPD+NSCLC specific genes corresponded to the PI3K/AKT, aryl hydrocarbon signalling and retinoic acid mediated apoptotic pathways.

Conclusions

After 4 weeks of osteogenic treatment, VICs demonstrated significant elevation in their alkaline phosphatase activity compare to the non-treated [control] group. Alkaline phosphatase and Von Kossa staining for calcium on fixed cells were also positive in treated cells. The protein expression of wnt3a and osteonectin were also increased in treated cells after 25 days.



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Anna Meredith

NORMALIZATION OF PROTEIN EXPRESSION IN THE MYOCARDIUM FOLLOWING MECHANICAL CIRCULATORY SUPPORT IN HEART FAILURE PATIENTS

Background/objectives

Management of the failing heart involves medical therapy, mechanical circulatory support (MCS) and heart transplantation. Unloading of the heart by MCS may induce reverse remodeling and normalize cardiac parameters (cardiac chamber geometry, size, volume, ejection fraction and fetal gene expression). We undertook to identify protein markers of heart failure and determine protein expression within the heart upon MCS. We examined the hypothesis that expression changes in the failing heart revert to a signature closer to that seen in healthy non-failing hearts following circulatory support with MCS.

Methods

Left ventricular (LV) apical tissue cores removed during LV assist device (LVAD) implantation, and corresponding explanted hearts obtained at the time of transplantation were examined. LV apical tissues at time of LVAD implant from 20 patients with a mean age of 51.3 ± 3.3 years were analyzed. Nine matched samples from patients who subsequently underwent transplantation were analyzed. Myocardial apical samples from non-transplantable healthy human hearts were used as a control. Serial sections were prepared with standard histology stains for quantification of myocardial fibrosis and remodeling. Immunohistochemical staining for protein markers of myocardial dysfunction and remodeling (galectin-3, versican, matrix metalloproteinases 2 and 9) was performed.

Results

Mechanical unloading of the LV induced changes in galectin-3, versican and MMP-2 and -9 expression levels closer to that observed in healthy myocardium.

Conclusion

Deleterious LV remodeling and alterations in contractile function in HF are associated with changes in gene and protein expression, including dysregulated expression of fibrotic markers. Unloading of the heart through MCA results in decreases in myocyte hypertrophy and myocardial fibrosis with concomitant changes in protein expression. In conclusion, gene and protein expression alteration is a function of mechanical unloading, and a time dependent alteration in relevant markers exists.

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Amanda Vanden Hoek

IDENTIFICATION OF A NOVEL COAGULATION FACTOR X COMPOUND HETEROZYGOUS MUTATION ASSOCIATED WITH DIFFERENTIAL INITIATING CLOTTING PATHWAY FUNCTION

Background/objectives

Factor X (FX) deficiency is a rare coagulation disorder characterized by a decrease in circulating FX antigen and/or activity levels, which can result in a variable bleeding diathesis.

Patient History: A propositus now aged 75 with a moderate bleeding diathesis is described. With prostate surgery, he had unexpected bleeding, that could not be explained surgically, requiring large volumes of plasma and red cell concentrates. Other surgical challenges, including dental extractions, were not complicated by bleeding but were preceded by plasma infusion.

Methods

Plasma FX antigen levels were assayed by Western blot using FX-specific monoclonal antibodies. Prothrombin time and activated partial thromboplastin time clotting tests were used to evaluate FXa activity in the extrinsic and intrinsic initiating branches of coagulation, respectively. The entire F10 gene (8 exons and flanking intronic sequences) was amplified using PCR and sequenced to identify mutations.

Results

DNA sequence analysis identified two heterozygous mutations. The first was a previously reported mutation that disrupts the splice site between exons I and II (IVS1 +1 G>A) and was hypothesized to lead to premature degradation of FX mRNA transcripts (Wang WB et al, Haemophilia 2005). This explains a 50% loss of antigen in our heterozygous patient. The second was a novel mutation at nucleotide 28145 (C>T) which results in an Arg386 to Cys (Arg386Cys) substitution in the serine protease domain. Quantification of plasma FX antigen by Western blot revealed 15% of normal, which correlated with 15% extrinsic pathway activity. However, intrinsic pathway clotting activity was only 5% of normal. The fragmentation of FX antigen in plasma after initiation of coagulation was followed over time. When initiated through the extrinsic pathway, the patient's FX fragmentation profile was identical to normal plasma. However, when clotting was triggered through the intrinsic pathway, activation to FXa and appearance of other fragments was notably slower. This further confirms that the patient's novel FX defect predominantly affects the intrinsic pathway while maintaining normal function in the extrinsic pathway.

Conclusions

We describe a compound heterozygous FX deficiency. The first mutation has been reported before (IVS1 +1 G>A) and accounts for 50% loss of FX antigen. The second FX mutation is novel and may result in alternate disulfide bond formation, in particular at the nearby covalent link between the heavy and light chains of FX. Interestingly, the differential effect of Arg386Cys on the extrinsic and intrinsic coagulation pathways suggests that Arg386 may be involved in the substrate recognition by the intrinsic Xase complex. As this complex functions to amplify coagulation, Arg386Cys may be predicted to most affect hemostasis under severe conditions such as surgery.

Chansonette Harvard[1,2], Christopher Dunham[4], Harald Hutter[5], Jie Pan[5], Jeanette Holden[6,7], Suzanne Lewis[1,3,7], and Evica Rajcan-Separovic[1-3]

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Chansonette Harvard

EXPRESSION PATTERNS OF EXPORTIN 1 (XPO1) IN THE NERVOUS SYSTEM OF ADULT AND DEVELOPING C. ELEGANS, MOUSE AND HUMAN

Background/objectives

We have previously described a microdeletion syndrome which includes neurological and neurodevelopmental abnormalities. The minimal critical region on chromosome band 2p15-16 includes the gene exportin 1 (XPO1) which mediates the export of proteins and mRNA from the nucleus and has been implicated in the control of a variety of cell processes (control of mitotic cell cycle, ribosome and miRNA biogenesis, and viral genome transport). XPO1 is a highly conserved gene with orthologues in a variety of species including humans, mice and C. elegans. Although there is much in literature describing the cellular function of XPO1, very little information exists regarding its expression pattern in the nervous system making it an interesting candidate gene for functional neuronal studies.

Aim: The aim of our study is to determine the expression pattern of XPO1 in the brain and other tissues in mouse, human and *C.elegans.*

Methods

We engineered fosmid-based reporter gene constructs containing XPO1 and GFP and used them to create transgenic C. elegans strains (VH2119 and VH2120) to study expression of XPO1. Immunohistochemistry using an XPO1 antibody was performed on adult mouse and human fetal and mature brain to determine expression patterns of XPO1.

Results

C. elegans transgenic animals: XPO1 is ubiquitously expressed in all tissues in transgenic animals. Earliest expression of XPO1 is seen in early gastrulation stage embryos and continues throughout embryonic and larval development into adult stages. Expression of XPO1 is enriched in the nuclei (typically excluding the nucleolus), but can also be seen in the cytoplasm. Expression of XPO1 is visible in neuronal nuclei and cytoplasm. Mouse organs: In the adult mouse, XPO1 shows wide cytoplasmic expression in the brain as well as in the gut, spleen, and lung. In human fetal brain, positivity was especially intense in some cells of the mitotically active immature ependyma (a pseudostratified epithelium) overlying the germinal matrix. The latter constitutes a dense stem cell population that sequentially gives rise to neuronal and glial precursors that eventually migrate out into the cerebrum. Mild positivity was also seen in Purkinje cells, inferior olive, substantia nigra and Cajal-Retzius cells in the cortex. In the mature human brain, mild positivity was seen only in Purkinje cells and inferior olive cells. The staining was predominantly nuclear, but cytoplasmic staining was also noticeable, particularly in cells undergoing mitosis

Conclusion

XPO1 may play a role in brain development based on its expression in the germinal matrix of the fetal human brain which has an important role in neurogenesis. Furthermore, its expression is noted in all stages of development for C. elegans and is widespread in the adult mouse brain. This gene may have a role in the neurodevelopmental abnormalities noted in patients with the 2p15-16 deletion.

Dana Kyluik [a,b,c], Wendy M. Toyofuku [b,c] and Mark D. Scott [a,b,c]

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

Dana Kyluik

IMMUNOCAMOUFLAGE OF ALLOGENIC CELLS: ALTERNATIVE POLYMERS TO METHOXYPOLY (ETHYLENE GLYCOL)

Background/objectives

The immunocamouflage of allogeneic donor cells (red and white blood cells) has primarily focused on the covalent grafting of methoxypoly(ethylene glycol) [mPEG] to cell membranes. The grafted mPEG dramatically reduces in vivo antigenic recognition and immunogenicity of donor cells. However, a small body of literature may argue against clinical use of mPEG. A novel group of polymers, polyoxazoline [POZ], may be an effective next generation alternative.

Methods

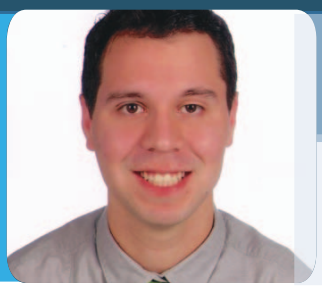
To assess the utility of POZ, human red blood cells (RBC) and peripheral blood mononuclear cells (PBMC) were modified with activated 20 kDa POZ or mPEG (0-4 mM; 60 minutes at pH 8.0). A two-phase separation system was used to quantify RBC membrane modification. RBC osmotic fragility was used to evaluate membrane stability. PBMC viability was assessed via 7- amino-actinomycin D (7-AAD) incorporation. The efficacy of immunocamouflage was measured by reduced detection of blood group antigens and PBMC CD markers via flow cytometry.

Results

Grafting of mPEG and POZ to RBC resulted in comparable 2-phase partitioning curves. mPEG showed improved partitioning over POZ at lower grafting concentrations (0.5 mM 36.2±6.1 vs 11.2±2.8%), while POZ displayed slightly superior partitioning at higher concentrations (2 mM 59.9±3.5 vs 73.2±5.0%). Osmotic fragility revealed no overall differences in RBC membrane stability while POZ demonstrated slightly elevated spontaneous lysis at 2 mM. Both polymers showed similar dose dependent decreases in PBMC CD3 and CD4 detection. At 2 mM, CD3 and CD4 detection was reduced 88% and 98% with mPEG and 86% and 97% with POZ.

Conclusions

This data clearly demonstrates that other polymers can effectively modulate the antigenicity and immunogenicity of allogeneic donor cells. Successful implementation of this technology may prove useful in preventing rejection of allogeneic blood cells and tissues.



Jaques Courtade

J.A. Courtade[1], P.C. Orban[1], C.B. Verchere[1]

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IMPAIRED PROCESSING OF PROIAPP AS A POSSIBLE CAUSE OF RAPID AMYLOID FORMATION AND GRAFT FAILURE IN HUMAN ISLET TRANSPLANTS

Background/objectives

Type 2 diabetes is marked by a progressive loss of pancreatic beta-cells that secrete insulin, resulting in poor blood glucose homeostasis. Islet amyloid polypeptide (IAPP), a hormone processed by enzymes PC2 and PC1/3, accumulates in pancreatic islets as a primary component of amyloid deposits. Formation of these deposits has been linked to type 2 diabetes pathogenesis and poor graft survival following islet transplantation. We aim to determine if accelerated amyloid formation, due to defective IAPP processing, contributes to early islet graft failure and type 2 diabetes.

Methods

To detect if IAPP is misprocessed in human islet transplant recipients and type 2 diabetic patients, we will develop a novel ELISA specific to IAPP precursors. We have taken three approaches to model defective IAPP processing in mice. In the first approach, we designed a PC2 conditional construct to selectively remove exon 4 of the Pcsk2 gene in mouse embryonic stem (ES) cells. Individual ES clones were selected for on media containing neomycin and ganciclovir. For the second approach, we will cross PC2 global knockout mice to human IAPP mice and correct for deficient glucagon processing by overexpressing PC2 in alpha cells. For our third approach we will transplant islets from PC2 knockout mice expressing human IAPP into diabetic NOD.scid recipients. In model diabetic mice and transplanted mice, we will perform glucose tolerance tests to identify early graft failure. Additionally, we will isolate pancreatic islets and transplanted grafts, and stain for amyloid formation, proliferative cells and apoptotic markers.

Results

We have identified a single homologous recombination event in which the floxed PC2 exon 4 cassette was inserted into the Pcsk2 genomic locus. This result was verified by PCR utilizing primers within the plasmid cassette and adjacent to the insertion site. Southern blot analysis confirms the presence of a 6.0 kb BglII digested fragment consistent with a correct recombination event.

Conclusion

We intend to demonstrate that IAPP intermediates contribute to increased amyloid formation and subsequently, graft failure in a transplant model. This work will pave the way for new drug treatments to target type 2 diabetes and early graft failure in islet recipients.

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Dhananjay Namjoshi

THE LIVER X RECEPTOR AGONIST GW3965 IMPROVES COGNITIVE DEFICITS AND PREVENTS ACCUMULATION OF ABETA IN WILD-TYPE MICE FOLLOWING REPETITIVE CLOSED HEAD INJURY

Background/objectives

Traumatic brain injury (TBI) is a major risk factor for dementia, particularly of Alzheimer's disease (AD). Traumatized brains produce large quantities of amyloid beta (Abeta) peptides that can accumulate in amyloid plaques similar to those found in the brains of AD patients. Many studies have underscored the important role of cerebral lipid metabolism, particularly of the cholesterol transporter ATP-binding cassette transporter A1 (ABCA1) and the major lipid carrier in brain, apolipoproteinE (apoE) in AD pathology and Abeta clearance. Agonists of Liver X receptor (LXR) induce transcription of ABCA1 and apoE, reduce Abeta levels and improve cognitive function in AD mice. The objectives of the present study were to establish a mouse model of closed head injury (CHI) and to determine whether treatment with the LXR agonist GW3965 improves functional and biochemical outcomes after repetitive CHI in wild-type (WT) and apoE-/- mice.

Methods

Male WT & apoE-/- mice at 4 months of age were subjected to repetitive CHI separated by 24 h using a weight drop device. Injured mice were randomly assigned to two study arms. Mice in the treatment arm received an intraperitoneal bolus of GW3965 30 min after the second injury followed by treatment of GW3965 compounded in rodent chow to result in an average dose of 15 mg/kg/day. Mice in the control arm received normal chow. Motor performance was assessed by the accelerating rotarod test at 1, 2, 7 and 14 day post-CHI. Cognitive function was assessed using novel object recognition test at 2, 7 and 14 day post-CHI. Endogenous Abeta 40 and 42 levels were quantified from the ipsilateral half brains of WT mice collected at 2, 7 and 14 day post-CHI.

Results

CHI induced significant motor deficits in WT and apoE-/- mice from both control and treatment arms within 24 h as indicated by shorter rotarod latencies. The degree of motor deficits in apoE-/- mice was significantly greater compared to WT following CHI. GW3965 treatment, however, failed to significantly improve motor performance of both WT and apoE-/- mice. CHI also impaired object recognition memory of WT and apoE-/- mice in both control and treatment arms 48h post-CHI. While WT mice in the control arm continued to exhibit memory deficit at 7 and14 days, GW3965 improved memory in WT mice 7 days after CHI. Conversely, GW3965 failed to improve CHI-induced memory deficits in apoE-/- mice at 7 and 14 days after injury. Repetitive CHI significantly increased Abeta 40 and 42 levels in the ipsilateral half-brains of WT mice within 24h, which were suppressed by GW3965 treatment.

Conclusions

Our preliminary results suggest that treatment with GW3965 may improve post-CHI cognitive function and prevent Abeta accumulation in WT mice. As expected, apoE deficiency leads to more severe motor dysfunction following head trauma. GW3965 treatment failed to improve CHI-induced cognitive deficits in apoE-/- mice, suggesting that apoE may mediate the beneficial effects of LXR agonists in facilitating recovery after CHI.

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Jianjia Fan

AN ABCA1-INDEPENDENT PATHWAY FOR RECYCLING OF A POORLY LIPIDATED 8.1 NM APOLIPOPROTEIN E PARTICLE FROM GLIA

Background/objectives

Within the central nervous system (CNS), lipids are transported on lipoproteins that circulate in the cerebrospinal fluid (CSF) and resemble peripheral high density lipoprotein (HDL) particles. Apolipoprotein E (apoE) is the major protein constituent of CNS lipoproteins and is secreted by astrocytes and microglia. We have previously shown that the cholesterol transporter ABCA1 is a key modulator of apoE levels and lipidation in the brain. In the present study, we investigated the biochemical nature of nascent apoE particles derived from primary WT and ABCA1-/- glia. We tested the hypothesis that apoE is secreted from glia using both ABCA1-depndent and independent pathways.

Methods

Primary murine mixed glia derived from WT and ABCA1-/- animals were conditioned with serum-free media for up to 96h. Non-denaturing and denaturing gel electrophoresis, immunoblotting and electron microscopy were used to characterize apoE particles in the conditioned media. Receptor-associated protein (RAP) was used to selectively inhibit LRP at 70nM, and inhibit both LRP and LDLR at 1uM in an apoE recycling study. ApoA-I at various concentration was used to stimulate the apoE recycling pathway.

Results

Here we show that apoE is secreted from wild-type primary murine mixed glia in at least five distinct nascent lipoprotein subspecies ranging from 7.5 to 17 nm in diameter. Negative-staining electron microscropy reveals rouleaux, suggesting a discoidal structure. KBr density gradient ultracentrifugation shows that all subspecies, except an 8.1 nm particle, are lipidated. Glia lacking the cholesterol transporter ABCA1 secrete only the 8.1 nm particles, which are poorly lipidated and nondiscoidal, but can accept lipids to form the full repertoire of wild-type apoE particles. RAP-mediated inhibition of apoE receptor function blocks the appearance of the 8.1 nm species, suggesting that this particle may arise through apoE recycling. Selective deletion of the LDLR reduces the level of the 8.1 nm particle production by approximately 90%, suggesting that apoE is preferentially recycled through the LDLR. Finally, apoA-I stimulates secretion of the 8.1 nm particles in a dose-dependent manner.

Conclusion

Our results suggest that nascent glial apoE lipoproteins are secreted through multiple pathways. One pathway requires ABCA1 and leads to the accumulation of several lipidated discoidal nascent apoE particles from 7-17 nm in diameter. The other pathway is ABCA1-independent but requires functional apoE receptors, primarily LDLR, and selectively regulates the secretion of a distinct 8.1 nm poorly lipidated apoE species, which is likely re-secreted following LDLR-mediated apoE uptake.

Xin Ye, Maged Hemida, Mary Zhang, Zhen Liu and Decheng Yang

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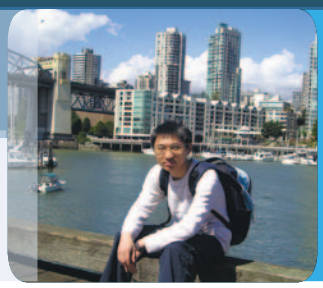


Xin Ye

microRNA (mir)-126 PROMOTES COXSACKIEVIRUS B3 REPLICATION BY ENHANCING THE ERK SIGNALING PATHWAY

Background/objectives

Coxsackievirus B3 (CVB3) is the primary pathogen of viral myocarditis, a life threatening heart disease in infants and children. To better understand the molecular pathogenesis of CVB3-induced myocarditis, we selected microRNs (miRNA), the recently discovered regulators of gene expression network, as the molecular determinant for this study. miRNAs regulate approximately one third of human genes and are involved in various physiological and pathological processes including viral infection. In this study we selected mir-126, an abundant miRNA regulating extracellular signal-regulated kinase (ERK) in cardiovascular endothelial cells, as the candidate for this investigation.

Methods

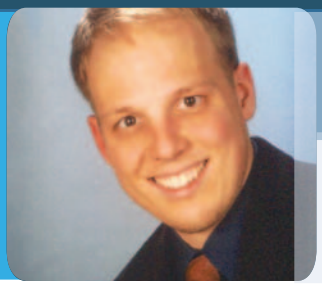
mir-126 mimics or negative control were transfected into HeLa and live cell numbers as well as mature mir-126 levels were measured. The transfected cells were then infected with CVB3 and the cell morphology were observed by microscopy. The effect of mir-126 on CVB3 replication was determined by Western blotting to detect viral protein and viral plaque assay to measure viral titer. The signal proteins involved in ERK pathway were also detected by Western blotting to elucidate the mechanism by which mir-126 regulates CVB3 replication. HeLa cells treated with ERK inhibitor (U0126) were used to confirm that the ERK activation is required for mir-126 mediated upregulation in CVB3 replication.

Results

Mir-126 lead to decreased number of live cells and significant cell elongation and membrane protrusions compared with control; ii). In CVB3 infected cells, mir-126 led to more cell detachment, caspase-3 activation, viral protein 1 (VP1) synthesis and viral progeny release (100 X increase); iv). mir-126 significantly enhanced the activation of ERK pathway during CVB3 infection by inhibiting its negative regulator, SPRED1; and v) the ERK inhibitor blocked the upregulation of CVB3 replication mediated by mir-126.

Conclusions

Mir-126 plays an important role in facilitating the replication of CVB3 through the ERK signal pathways.



Stefan Kommoss[1], Leah Prentice[1], Andrew McPherson[1], Karey Shumansky[1], Winnie Yang[1], Nataliya Melnyk[1], Janine Senz[1], Sohrab Shah1, David Huntsman[1]

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Stefan Kommoss

NEW GENE FUSION IDENTIFIED BY NEXT GENERATION GENE SEQUENCING IN YOLK SAC TUMOR OF THE OVARY

Background/objectives

Yolk sac tumor of the ovary is a rare but highly malignant germ cell neoplasm. Due to low case numbers it is difficult to study this entity in a clinical series, therefore little is known about its oncogenesis. The development of improved diagnostics and ultimately new therapeutics is hindered subsequently. It was the aim of this project to study yolk sac tumors using next generation sequencing techniques with the goal of identifying pathognomonic changes.

Methods

Next generation sequencing (NGS) using Ilumina GAII technology was used to analyze the transcriptome of 8 Flash frozen yolk sac tumors as collected by our ovarian tissue bank. Putative gene fusions were predicted using deFuse bioinformatics script and validated by Sanger sequencing. Fluorescence in situ hybridization (FISH) was used to further assess the affected gene loci.

Results

A total of 376 fusions were found among the 8 cases as revealed by NGS and the deFuse script. Nineteen of these fusions were in frame and of particular interest was the novel CREBBP-PARN fusion, hence it was selected for further investigation. The CREBBP-PARN fusion was confirmed by Sanger sequencing. In addition to the gene fusion the CREBBP flanking region is deleted in this case as shown by FISH.

Conclusion

Our current finding in yolk sac tumors is of great interest and may lead us towards the identification of a so far unknown pathomechanism in this rare malignancy. To further study this distinctive feature we have now broadened our approach to study a higher number and wider range of possibly affected germ cell tumors.

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Liat Apel-Sarid

NON-IMMUNE HYDROPS FETALIS IN VANCOUVER: ARE WE DIFFERENT?

Background/objectives

Analyze the causes of non immune hydrops fetalis (NIHF) in fetal autopsies at the British Colombia Children's and Women's Hospital (BCCW) in Vancouver, Canada.

Methods

A retrospective review of prenatal autopsies diagnosed with NIHF between 2006-2010 in the department of Pediatric Pathology at BCCW was done to determine identifiable causes for the hydrops.

Results

Among 2018 autopsies conducted in BCCW between 2006-2010 we found 87 cases with NIHF (4.3%).The cause for the NIHF was identified in 92% of cases, leaving 7 cases undetermined. The most prevalent cause for the NIHF was fetal chromosomal abnormalities, identified in 51cases (58.6%). The most common chromosomal abnormalities included Monosomy 45 (n=26), and Trisomy 21 (n=15). Other chromosomal abnormalities included: 6 cases of Trisomy 18, 3 cases of Trisomy 13 and a single case of del 5p (cri-du-chat syndrome). Cardiovascular abnormalities in otherwise normal karyotype fetuses were identified in 5.7% of cases (n=5). Parvovirus B19 infection was confirmed in 3 cases. Other causes included: Twin-to-twin transfusion syndrome, multiple pterygia, adenomatoid cystic malformation, diaphragmatic hernia, metastatic congenital neuroblastoma, fetal gonadoblastoid dysplasia and inferior vena cava thrombus.

Conclusions

Only 8% of cases did not have an identifiable cause for the hydrops, which is lower than what has been reported in previous studies. A chromosomal abnormality was identified in 58.6% of cases, which is higher than what has been previously reported. The most common cause of NIHF in this series was fetal chromosomal abnormalities. The incidence of cardiac malformations in our series is lower than previously published, and is likely related to the much higher rate of chromosomal abnormalities detected.

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Gulisa Turashvili

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

Background/objectives

RNA extracted from paraffin blocks can be used for clinical molecular diagnostic assays, including RT-PCR, quantitative PCR, cDNA library construction and most recently whole transcriptome shotgun sequencing. Although the capacity to extract high quality RNA from paraffin blocks is crucial, there is little information available to guide laboratories in their selection of tissue fixation, processing and RNA extraction techniques.

Methods

Human tissue samples (three each of colon, liver and muscle) were subjected to the following fixation and processing conditions: (1) flash freezing; (2) neutral buffered formalin (NBF) fixation <24 hours (NBF24); (3) NBF fixation 7 days (NBF7); (4) molecular fixative (MF) <24 hours; (5) MF 7 days. NBF-fixed samples were processed by standard processing (Tissue-Tek VIP5 processor, Somagen, Canada), and MF-fixed samples by rapid processing (Tissue-Tek Xpress, Somagen). Total RNA was extracted using phenol-chloroform manual extraction, RecoverAll (Ambion, USA), WaxFree RNA (Trimgen, USA), and RNeasy FFPE Kit (Qiagen, Canada). RNA was quantified using a Nanodrop spectrophotometer, and one-step RT-PCR was used for the amplification of two ACTB fragments (621 bp and 816 bp).

Results

Manual extraction and WaxFree RNA kit yielded higher amounts of RNA when compared with RNeasy and RecoverAll kits, independent of the type of tissue, fixation or processing (Fig. 1). For frozen tissues, there was no difference between manual extraction and WaxFree RNA kit for 621 bp amplicon but manual extraction performed better for 816 bp amplicon ($p<0.001$). In MF-fixed tissues, both amplicons were successfully amplified using all extraction methods except for WaxFree RNA kit. For NBF24 tissues, 621 bp amplicon was amplified using all kits but manual extraction performed better for the 816 bp amplicon ($p<0.001$). For NBF7-fixed tissues, manual extraction and WaxFree RNA kit were superior to RNeasy and RecoverAll kits ($p<0.01$). None of the extraction kits succeeded in amplifying the 816 bp amplicon in a majority of NBF7 samples.

Conclusion

The molecular fixative, regardless of the duration of fixation, and the rapid processing system used in this study were able to preserve RNA in paraffin blocks with successful RT-PCR for amplicons as long as 816 bp, making these techniques suitable for use in downstream molecular diagnostic assays.

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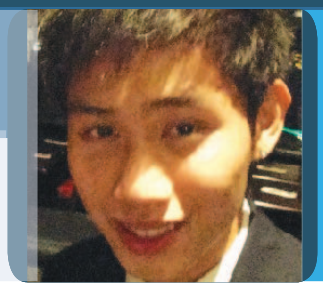


Arkhjamil Angeles

THE ROLE OF SERUM RESPONSE FACTOR IN COXSACKIEVIRAL CARDIOMYOPATHY

Background/objectives

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

Methods

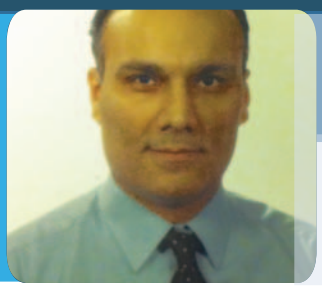
Cell lysates were collected from murine cardiomyocytes and A/J mice infected with CVB3 for different time courses. SRF protein and gene expression were examined by immunoblotting and real-time qRT-PCR, respectively. To determine SRF cleavage by CVB3 proteases, HeLa cells were transfected with Flag-tagged SRF followed by CVB3 infection or co-transfection with 2Apro or 3Cpro plasmids. SRF expression was analyzed by immunoblotting. Furthermore, deletion and point-mutation SRF constructs were applied to identify the cleavage site. In addition, immunocytochemistry was performed to examine intracellular localization of SRF fragments. The transactivation function of SRF cleavage products was determined by luciferase assay using a reporter construct.

Results

CVB3 infection of mouse cardiomyocytes resulted in a decrease in SRF protein expression, but not in mRNA expression. Further investigation by antibody epitope mapping demonstrated that SRF was cleaved into 47kDa and 20kDa fragments after CVB3 infection. We further showed that 2Apro expression led to similar fragmentation of full-length SRF. The cleavage site was identified at the 327 amino acid position. Confocal microscopy showed a differential localization of the SRF fragments, where SRF-N resided exclusively in the nucleus and SRF-C was mainly in the cytosol. In addition, these truncated SRFs lost their transactivation function as demonstrated by luciferase assay. SRF-N also exhibited dominant negative competitor activity for SRF-associated transcription.

Conclusions

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



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Ehsan Davani

ERYTHROPOIETIN IMPROVES CARDIAC FUNCTION IN A MURINE MODEL OF SEPSIS

Background/objectives

Left ventricular dysfunction during septic shock contributes to the poor prognosis. The serum erythropoietin level or response is lower in septic and critically ill patients. We tested the hypothesis that erythropoietin alters left ventricular contractility in a lipopolysaccharide (LPS) animal model of sepsis.

Methods

The effect of erythropoietin was investigated in two models (A: in vivo and B: ex vivo) of CD-1 mice. A: Six hours after injection of LPS, volume conductance micro-catheter was inserted into the heart. Cardiac contractility was measured using pressure volume loops in the presence or absence of erythropoietin B: Using Langendorff system, hearts were exposed to 20 minutes global ischemia and one hour reperfusion in the presence or absence of erythropoietin. Left ventricular pulse pressure and heart rate were measured. Cardiac sections and protein lysis were prepared to evaluate protein expression by confocal microscopy and Western blotting respectively.

Results

LPS injection increased heart rate and decreased end systolic elastance and ejection fraction ($p< 0.05$). Erythropoietin increased ejection fraction and end systolic elastance to 58.3 ± 2.6% and 31.6 ± 9.4 respectively. Erythropoietin also significantly improved left ventricular pulse pressure (23.6 ± 2.3) and heart rate (244 ± 21) in the ex vivo model. This effect of erythropoietin is through the activation of Jak-STAT pathway, expression of Bcl XL and Akt and reduction of ICAM-1 protein expression.

Conclusion

Erythropoietin protects left ventricular performance in the LPS model of sepsis in mice through activation of Jak-STAT pathway and modulation of ICAM-1 in cardiac tissue.

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Jayoung (Janet) Lee

SOMATIC MUSCLE MITOCHONDRIAL DNA MUTATIONS IN HIV-INFECTED PATIENTS WITH CHRONIC PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA (CPEO)-LIKE SYNDROME

Background/objectives

Chronic Progressive External Ophthalmoplegia (CPEO) is a mitochondrial (mt) disorder (~1 in 8500 people) primarily affecting the extra-ocular muscles, usually due to mtDNA damage or polymerase gamma (POLG) gene mutations. It was recently suggested that a CPEO-like syndrome seen in HIV-infected patients may be akin to an acquired form of the disease. It is known that antiretroviral drugs used to treat HIV can affect mitochondrial function. The objective of this study was to investigate the muscle mtDNA mutation burden in such HIV-infected patients with CPEO-like disease, as well as non-HIV-infected individuals.

Methods

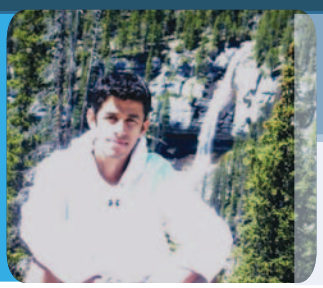
We studied skeletal muscle biopsies from HIV-infected patients with CPEO-like syndrome (N=3), HIV-negative CPEO patients (N=8), and age and muscle type-matched (2:1) non-CPEO individuals (N=21) who underwent muscle biopsies to investigate possible myopathy but showed no pathology. Total DNA was extracted from muscles biopsy/autopsy tissues, and amplified by long PCR to detect mtDNA deletions. The whole mtDNA genome was also sequenced to identify mtDNA mutations that may not be detected by standard of care testing. For the detection of somatic mtDNA point mutation burden, a portion of the mtDNA D-loop (509 bp) was amplified and cloned into a plasmid. Eighty individual clones were then re-amplified and sequenced. All sequences were compared to the individual's consensus sequence to calculate the mtDNA mutation burden. In some instances, muscle tissue from the affected eyelid will be compared to that of the quadriceps from the same individual.

Results

As the investigators are still blinded to the identity of the samples, it is too early to report results on the specific individuals. However, preliminary results indicate that for the 14 individuals analyzed to date, the muscle mtDNA mutation burden ranges from 3 to 24 mutations per 80 clones. This is well above the assay background rate caused by PCR enzyme (pfu) errors, which was determined twice for a plasmid internal control to be 1 mutation per 80 clones.

Conclusions

The large difference between the number of mtDNA mutations observed in our samples relative to that of the internal control suggests that this mtDNA mutation burden assay will be a useful tool to measure and compare somatic mtDNA mutations in muscle tissue.



Mehul Sharma

Andrea Tam*, Mehul Sharma*, Jayoung J. Lee, Beheroze Sattha, Hélène Côté, Andre Mattman

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ANALYSIS OF MITOCHONDRIAL DNA MUTATIONS IN PATIENT SAMPLES SUSPECTED OF HAVING MULTIGENERATIONAL MITOCHONDRIAL DISEASE

Background/objectives

Defective mitochondrial replication or function, can lead to impaired oxidative phosphorylation and mitochondrial dysfunction. Mutations and deletions in the mitochondrial DNA (mtDNA) are major causes of mitochondrial diseases. Although certain mtDNA mutations develop with age and oxidative stress, other mtDNA defects can be maternally inherited. In this study we analyzed quadriceps muscle biopsies from two unrelated patients suspected of having multigenerational mitochondrial disease characterized by fatigue, myopathy and decreased mitochondrial complex IV activity. Routine mtDNA tests failed to identify any defect that could explain their disease. Using the techniques described below, we further investigated the muscle samples for mutations or rearrangements that may be responsible for the clinical phenotype of these patients. Furthermore, blood samples from one patient and from her extended family members are currently being investigated.

Methods

DNA was extracted from the two quadriceps muscle samples. The amount of mtDNA relative to nuclear DNA (mtDNA/nDNA ratio) was determined by qPCR. Large rearrangements were qualitatively analyzed by long PCR on the mtDNA followed by agarose gel electrophoresis. Finally, point mutations or small rearrangements on the mtDNA were studied by sequencing the long PCR products. The same experiments were carried out on the blood samples.

Results

The first sample studied (pt1) showed no mtDNA alteration. The second sample (pt2) on the other hand, had low mtDNA/nDNA ratio (1505) compared to pt1 (3198) or to controls (mean ± SD 3088 ± 1543: panel of 10 quadriceps biopsies that showed no mitochondrial pathology). The long PCR also showed a ~4 kb mtDNA deletion affecting a small percentage (~10%) of the mtDNA. A potentially clinically relevant insertion at position nt194 within the non-coding regulatory D loop region was present in 35-40% of the mtDNA extracted from muscle of pt2. Preliminary analysis of blood samples from pt2 and one sibling showed no signs of this mutation.

Conclusion

The insertion seen in pt2 is very close to the mtDNA origin of replication and may affect mtDNA replication and/or transcription. The insertion could therefore be consistent with the low levels of mtDNA in pt2's quadriceps muscle biopsy. The fact that the insertion not being seen in pt2's blood is intriguing. However, it is common to detect mutations in skeletal muscle that are absent in blood cells from the same individual. Current work includes determining the exact heteroplasmy for the nt194 insertion, as well as possibly obtaining muscle tissue from other relatives. The identification and characterization of a disease causing mutation would clarify the diagnosis and thus be of benefit to the families.

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Adam Ziada

DEVELOPING A NEXT GENERATION SEQUENCING-BASED ASSAY TO QUANTIFY MITOCHONDRIAL DNA MUTATION BURDEN

Background/objectives

It has been suggested that nucleoside reverse transcriptase inhibitors (NRTIs) used in the treatment of HIV infection, can cause errors in the replication of mitochondrial DNA (mtDNA). This could be due to oxidative stress or a decrease in mitochondrial polymerase gamma fidelity. Either could result in an accumulation of point mutations in the mtDNA. Our laboratory previously developed an assay to analyze mtDNA mutation burden. This assay involved amplifying a small section of the mtDNA D-loop (a non-coding regulatory region), inserting it into a plasmid, isolating and sequencing 80 individual clones per sample. By comparing each clonal sequence to the sample's consensus sequence, a measure of mtDNA mutation was obtained. The above assay is costly, both in time and expense. Furthermore, PCR errors caused a high background to signal ratio. To address this problem we will take advantage of next generation sequencing (454 or GS Junior, Roche) and an approach recently described by Jabara et al, 2011.

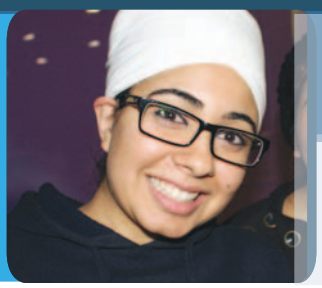
Methods

For each sample studied, total DNA (10,000 mtDNA copies) will be mixed with an internal control plasmid (5000 copies) that contains a similar but easily distinguishable DNA sequence. The primers used to amplify the mixture will contain, in addition to the mtDNA D loop-specific sequence, a tag to identify the sample, followed by 10 degenerate (N) bases, and a forward or reverse primer sequence. After one round PCR amplification, the product will be purified and re-amplified with the F+R primers, then subjected to deep sequencing, generating approximately 3000 reads per sample (2000 from the sample template, 1000 from the internal control plasmid). The degenerate bases will result in over 60 000 unique "IDs" that will identify the products of the first round of PCR, such that PCR errors can be distinguished from template mutations. Furthermore, the internal control plasmid will allow the determination of the first round PCR error rate. Afterward, the prevalence of "true" somatic mtDNA mutations will be calculated by aligning and comparing each template sequence to the consensus mtDNA sequence of the given individual.

Relevance

he accumulation of somatic mtDNA mutations is implicated in the aging process. Anything that accelerates this may accelerate the aging process. This assay will facilitate the study of acquired mtDNA mutations, something that is technically challenging given the naturally heteroplasmic nature of mtDNA.

Jabara C, Jones C, Anderson J, Swanstrom R. CROI 2011, abstract #665.



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Gurmeet Kaur Sohi

OPTIMIZING AN ASSAY TO MEASURE MITOCHONDRIAL DNA OXIDATIVE DAMAGE

Background/objectives

A published assay developed by the Santos group quantifies lesions to mitochondrial DNA (mtDNA) resulting from oxidative damage. The assay amplifies two large fragments of mtDNA and exploits the fact that mtDNA damage can slow down/inhibit the progression of PCR polymerase, leading to less efficient amplification and thus less PCR product. Lesion frequency can be mathematically quantified based on the relative amplification of long PCR products. We sought to adapt, optimize, and validate this assay using in-house tools. We aim to later quantify mtDNA oxidative damage resulting from exposure to HIV antiretroviral drugs.

Methods

Three DNA quantification methods were compared for their accuracy and reproducibility in quantifying initial PCR template: PicoGreen fluorescence quantification, NanoDrop spectrophotometer A260, and SYBR Green qPCR on a short D-loop region of mtDNA. Human hepatocellular carcinoma cells (HepG2) were exposed to hydrogen peroxide (H2O2) at a concentration of 17 M for 2 hours or 0.2 M for 1 hour followed by recovery in medium for 0, 15, 30, and 60 min, to allow mtDNA repair. The DNA was then extracted and subjected to the assay. To assess the applicability of the assay to clinical samples, human skeletal muscle DNA samples (n=44) were also assayed.

Results

The PCR products for which the template was initially quantified by NanoDrop (CV=6.5%) and qPCR copy number (CV=7.0%) showed low variability in agarose gel intensity, implying that the initial template quantification was more reproducible. A greater variability was observed for template quantified by PicoGreen (CV=21%). DNA from hydrogen peroxide-exposed cells showed decreased amplification of long PCR product. With longer repair time, the amount of PCR product increased and lesion frequency decreased. The relative lesion frequency post H2O2 exposure was 3.2 and improved down to 1.3 after 60 min allowed for repair. The clinical samples showed a wide range of relative lesion frequencies (0.0 to 3.9).

Conclusion

The preferred method for quantification of template DNA was short D-loop copy number by qPCR as it eliminates the need to later normalize the data to initial copy number of mtDNA. Although NanoDrop determination also had low variability, it cannot be used on unpurified PCR products, limiting its usefulness. Following H2O2 exposure, oxidative damage and repair was readily detected with the assay. The fact that clinical samples yielded a broad range of relative lesion frequency, and that these were higher than for the undamaged control DNA, bodes well for its use in investigating the effects of antiretroviral drugs on mtDNA oxidative damage in human tissues and cell models.

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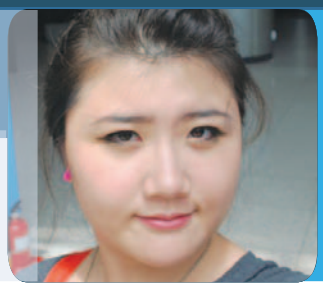

Aini Zhou

PROTEIN SIALYLATION IN THE DEVELOPING RAT BRAIN

Background/objectives

Sialic acid (SA) is a nine carbon sugar found on the terminal of carbohydrate chains of many glycoproteins located on cell surfaces. The most common form of SA is N-acetyl-neuraminic acid (Neu5Ac or NANA). Plasma and brain levels of SA are decreased in neonates fed formula diets lacking SA, with the speculation that the enzymatic pathway for SA synthesis is immature at birth. Therefore, it is hypothesized that dietary SA plays a vital role in neural development during early life. The overall objective of an ongoing project in our group is the study of lipid and protein sialylation during early development and the impact of SA content in the diet. Here, we analyzed lipid- and protein bound SA in the brain from birth throughout nursing. The next objective is the use of differential proteomics for the identification of specific glycosylated proteins, and the assessment of their sialylation progress during development.

Methods

Brain was homogenized, the lipid and protein fractions separated, and SA concnetrations analyzed employing a 2-thiobarbituric acid assay. Protein extracts were prepared for two-dimensional sodium-dodecylsulfate polyacrylamide gel electrophoresis (2D-SDS-PAGE). Coomassie Blue was used for gel staining. Gels were scanned and compared using PDQuest software (BioRad). Selected protein spots were excised from gels for identification. Excised gel pieces underwent in-gel digestion with bovine trypsin, and extracted peptide mixtures were analyzed using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). Peptide maps were submitted to MASCOT software (www.matrixscience.com) and the SwissProt database (ca.expasy.org) for protein identification.

Results

SA shows a continuous increase over the first 19 days of life, for both protein and lipid bound fractions. Employing narrow pH range (pH 4-7) IPG strips enabled an effective separation of complex protein mixtures from brain extracts using 2D SDS PAGE. Employing mass spectrometry compatible gel stains, we have identified several key proteins directly from 2D gels. The next steps is the specific staining of glycoproteins and the analysis of the sialylation extent during development.

Conclusions

Sialylation of lipids and proteins in the brain increases during early development, yet little is known of the affected individual lipids and proteins. We have successfully developed and started to apply advanced methods that allow the effective study of the specific roles of sialylation, and the effect of dietary SA supply on the brain during early development.

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Jenny Hanbi Kim

SINGLE EXPOSURE TO GHB - DOES INTRAINDIVIDUAL VARIATION IMPACT THE INTERPRETATION OF LEVELS IN HAIR?

Background/objectives

Gamma hydroxybutyrate (GHB) and its analogs are drugs of abuse that are sold as "dietary supplements" for purported health effects including bodybuilding, weight loss, and sleep. It is notorious because of its association with drug facilitated sexual assault (DFSA). Segmental hair analysis might assist in the verification of an exposure to a single dose. Because GHB is also an endogenous compound levels need to be discriminated. In one of our cases of suspected DFSA we were prompted to what appeared to be a cyclic re-occurrence of elevated levels of GHB with a cycle time of about 6 to 8 weeks. The peak levels were within the range of endogenous levels, but the trough levels considered as individual baseline level were below the limit of quantitation (LOQ of 0.1 ng/mg). The question was raised if those cycles can occur naturally. The purpose of this study was to establish whether or not the intraindividual variation of GHB levels in scalp hair is large enough to falsely indicate the exposure to GHB, e.g. in a suspected DFSA.

Methods

Hair samples were cleaned with acetone, hot water, and dichloromethane prior to segmentation. The samples were digested using 1N NaOH, and GHB was isolated using liquid-liquid extraction. (Liquid Chromatography/Mass Spectrometry) LC/MS/MS was performed using atmospheric pressure chemical ionization in the negative mode, multiple reaction monitoring and deuterated standard (GHB-D6). 140 segments from 14 individuals (5 males and 9 females) with no known exposure to GHB were analyzed reflecting the processing of real cases after a single exposure.

Results

Results from one female case had to be excluded because no numerical values were obtained. For all those samples that gave results greater than the LOQ, the intraindividual range was 11.9 to 53.9% relative standard deviation.

Conclusion

This study investigated the question if an elevated level of GHB in a segment can be caused by intraindividual variation. The data showed that in the specimens of 8 females and 5 males, the intraindividual variation observed was larger than indicated by a paper published earlier. However, it was not large enough to compromise the interpretation of the underlying case. The case was reported as "likely multiple exposure".

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Sherry Lee

EFFECT OF POLYCOMB REPRESSIVE COMPLEX 2 CONTAINING EZH2 TYROSINE 641 MUTANTS ON THE METHYLATION OF LYSINE 27 IN HISTONE 3

Background/objectives

Enhancer of zeste homologue 2 (EZH2) is the enzymatic subunit of the Polycomb repressive complex 2 (PRC2). EZH2 functions as lysine methyltransferase which performs the addition of methyl groups to lysine 27 on Histone 3. Increased H3K27me3 levels, in solid tumours, correlates to EZH2 over-expression. In lymphomas, somatic point mutation of Tyrosine 641 in the enzymatic SET domain is selected for, where tyrosine is replaced by phenylalanine, asparagine, histidine, or serine. This mutation is found in 22% of germinal center B-type diffused large B cell lymphoma (DLBCL) cases. The EZH2 Y641X mutant is dependent on the wild type activity performing the mono- and di-methylation, and predominately tri-methylates the already methylated target histone lysines thereby explaining why it is heterozygous. It is known that localized tri-methylation of lysine 27 induces epigenetic gene repression via chromosome condensation. Although EZH2 targets are not yet fully characterized, we hypothesize that EZH2 mutation contributes to lymphogenesis by the repression of genes involved in the control proliferation and differentiation.

Our objective is to explain the functional mechanism of this gain-of-function mutant phenotype. This involves asking questions, such as, is there an increased ability for the mutants to recruit essential components of the PRC2 complex? We also hypothesize that perhaps the CDC2 pathway can differentially regulate the wild type and mutant EZH2.

Methods

We prepare N-terminal GFP-tagged wild type or mutant EZH2 transcript-containing plasmids, under the control of the CMV promoter. HEK293T cells are transfected at 40ng/cm(squared) with purified DNA plasmids. Plasmids containing only GFP-V5 are used as transfection control. Cells are harvested at 72hrs after transfection, and cell nuclear extracts are collected for Western blotting. Proteins or methylation levels are detected using standard Western Blot protocol and chemo-luminescence. The extent of PRC2 complex formation of the mutant EZH2 is assayed via immune-precipitation using GFP-Trap.

Results

As done in previous studies, we observe increase in H3K27me3 level in EZH2 Y641X DLBCL cell lines when compared to those that are wild-type, as well as in patient tissues (sample size =10). We also observed the same increase using in vitro transfection of wild type and Y641X plasmids with HEK293T cells.

In literature, we find that CDC2 modulates the formation of PRC2 complex via phosphorylation of EZH2. We test this relationship using a dominant negative CDC2 construct in our HEK293T cell model, and attempt to answer whether the EZH2 Y641X requires the formation of PRC2 for its activity.

Conclusions

Further investigation is underway to examine targets of EZH2, and to explain the mechanism of the increased activity of EZH2 Y641X, which plays a significant role in cancer development in DLBCL patients.

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Ananta Gurung

WT-1/ ER EXPRESSION IN ASCITIC FLUID AND PERITONEAL LAVAGE CYTOLOGICAL PREPARATIONS AND SURGICAL RESECTION SPECIMENS IN OVARIAN SEROUS CARCINOMAS

Background/objectives

The combination of Wilms Tumour-1 (WT-1) and Estrogen Receptor (ER) protein expression has been used in the identification of serous ovarian carcinomas. The presence of malignant cells in ascites or peritoneal washings may upstage disease. The aim of this study was to assess WT-1 and ER expression in ascitic fluid and peritoneal lavage specimens compared to the corresponding surgical pathology specimen.

Methods

All cases of serous ovarian carcinomas diagnosed in cytological preparations and surgical resections specimens at the Vancouver General Hospital over a one year period were identified. A total of 26 cases were reviewed. Additional sections from surgical resections and cell block cytological preparations were stained for WT-1 and ER expression. Intensity (0 = minimal to absent, +1 = moderate, +2 = intense), and percentage of positive cells (0 = 0-0.1%, 1 = 0.1-33%, 2 =34-66%, 3 = 67-100%) were recorded.

Results

Tumour cells were not identified in recut sections in one surgical specimen, and an insufficient number of tumour cells were seen in one cytology case for ER expression, so statistical analysis was performed on the remaining 25 (WT-1) and 24 (ER) cases. Both WT-1 and ER showed zonality of staining in tissue sections (well fixed/ peripheral portions of tumours stained more intensely) and were not affected by adjuvant chemotherapy (6 cases). Expression of WT-1 and ER was absent in the cytological specimen in 5/25 (20%) and 7/23 (30%) of cases respectively, where there was positivity in the surgical specimen. Cytological preparations were less sensitive than tissue sections in assessment of expression for both WT-1 and ER; however, this difference was statistically significant only for ER (p=0.023; WT-1 p = 0.35).

Conclusion

The absence of WT-1 and ER positive malignant cells in ascitic fluid and peritoneal lavage specimens should be interpreted with caution in patients who are suspected to have a serous ovarian carcinoma as there can be false negative results.

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