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Poster Presentations from the 2018 Maine Medical Center Research Institute (MMCRI) Summer Student Research Program

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Poster Presentations from the 2018 Maine Medical Center Research Institute (MMCRI) Summer Student Research Program

The following posters were presented as part of the 2018 MMCRI Summer Student Research Program. This program offers undergraduates and medical students a unique opportunity to conduct research in diverse clinical and biomedical science fields during the summer months. During the paid ten-week program, students participate in mentored independent research projects either in our state-of-the-art research facility, or working with physicians in a hospital setting to impact patient care or the outcome of treatment. Students also attend lectures and workshops featuring topics including bioethics, animal use in biomedical science and scientific presentation skills, and have the opportunity to attend presentations by guest scientists and MMCRI faculty. All students give a final presentation, which in 2018 involved a three minute oral presentation called a “Three Minute Thesis” as well as a scientific poster presentation. All authors have an affiliation with MMCRI, unless otherwise noted.
Multiple Myeloma (MM) is the second most common type of hematological cancer, formed from a series of somatic mutations to the plasma cells of the bone marrow (1). Initially patients respond well to chemotherapeutic treatment, but almost all eventually develop resistance to these treatments and experience relapse.

MM elicits in the unique and complex bone marrow microenvironment. Also, within the bone marrow are bone marrow adipocytes (BMAs) that form bone marrow adipose tissue and account for 50-70% of the total bone marrow volume. It is believed that BMAs provide a source of energy that aids in multiple myeloma cell metastasis (2).

Fatty acid oxidation is a process by which cells convert long-chain fatty acids into NADH, FADH2, and ATP in the mitochondria. (CPT1) is a transport enzyme in the outer mitochondrial membrane that transports long chain fatty acids into the inner mitochondrial space. It is the first, rate limiting enzyme of the carnitine system and subsequently of fatty acid oxidation (3).

Eto (etomoxir) is a pharmacological irreversible inhibitor of CPT1, effectively inhibiting fatty acid oxidation.

In other cancers, such as breast and prostate cancer, inhibiting fatty acid oxidation with the use of etomoxir has been proven to reduce cancer cell viability and proliferation.

Recently, etomoxir has been shown to have off target effects by inhibiting complex one of the electron transport chain at high dosages (4).

In addition to a potential energy source, BMAs have been shown to increase MM’s resistance to chemotherapeutic treatments (5).

We are examining the effect of etomoxir on different MM cell lines and if it increases MM sensitivity to other chemotherapeutic drugs.

Objective and Aim: An in vitro investigation at the effects of inhibiting CPT1 in MM cells and to design a drug combination treatment that effectively reduces MM cell viability.

Hypothesis: Inhibiting fatty acid oxidation in multiple myeloma cell lines will reduce cell viability and increase their sensitivity to other chemotherapeutic drugs.

**RESULTS**

**2. In vitro Combination Treatment of Etoomoxir and Bortezomib**

**A** OPM2: Etoomoxir + Bortezomib

<table>
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<th>Eto 0 µM</th>
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**B** MM1R: Etoomoxir + Bortezomib

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Cell viability of (A) OPM2 and (B) MM1R cells treated with etomoxir (eto) at 0 µM, 5 µM or 12.5 µM, in addition to various doses of bortezomib (bort), specifically 0 nM, 0.25 nM, 1 nM or 5 nM. Bioluminescence was used to measure tumor cell number and read 72 hours after the drugs were administered. OPM2 cells were seeded at 20,000 cells/well in 96-well plates. **p<0.0001, ***p<0.001.

**3. In vitro Combination Treatment of Etoomoxir and Dexamethasone Co-Cultured with MSCs**

MM1S cells were directly co-cultured with mouse mesenchymal stem cells (mMSCs) for 24 hours before etomoxir (Eto) and dexamethasone (Dex) were administered. The mMSCs were seeded at a cell density of 7500 cells/well. The MM1S cells were seeded at a cell density of 5000 cells/well. Statistical analysis was done using one way ANOVA test. *p<0.05, ***p<0.0001.

**CONCLUSIONS**

1. We have seen a decrease in cell viability in our in vitro models with the MM1S, MM1R, and OPM2 multiple myeloma cell lines when treated with etomoxir only.

2. Our preliminary data showing that etomoxir decreased cell viability, suggests that multiple myeloma may utilize fatty acid metabolism as a source of energy.

3. Combination treatments of etomoxir with other anti-myeloma drugs, such as bortezomib and dexamethasone showed a significant decrease in cell viability when compared to the control in MM1S and MM1R cell lines.

4. While we are seeing a trend in a reduction of cell viability with the use of etomoxir in all cell lines, more research is required to confirm this data and refute any claims of off target effects.

**FUTURE DIRECTIONS**

1. Isolate mitochondria to run a Seahorse assay to determine the dose of etomoxir that results in off target effects on the electron transport chain.

2. Determine the exact doses of etomoxir in combination with bortezomib and dexamethasone that are most effective in multiple myeloma cell lines.

3. Seed myeloma cells in direct, or indirect, co-culture with bone marrow adipocytes to investigate whether etomoxir has a greater negative effect on cell viability in a high lipid environment.

4. Create a CPT1 knockdown model using lipofectamine and siRNA to see the effect of complete inhibition of fatty acid oxidation without the risk of off-target effects.

5. Treat multiple myeloma cells with a combination of etomoxir and oralistat (fatty acid synthesis inhibitor) to try to reduce cell viability and to determine the role of lipids in myeloma cells.

6. Create a drug combination therapy including etomoxir, oralistat, and anti-myeloma drugs.

**REFERENCES**


Loss of miR-199b promotes expansion of myeloid-committed progenitors differentiated from bone marrow and spleen HSCs

Aidan McGroty1, Aldona Karaczyn1, Edward Jachimowicz1, Pradeep Sathyanarayana1
1Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, Maine

Abstract

Background: Perturbed hematopoietic stem cell (HSC) hematopoesis can cause blood cancers including acute myeloid leukemia (AML). To avoid this, HSC proliferation, survival and differentiation must be properly balanced. There is emerging evidence that microRNAs (miRNAs) might be well suited to regulate these decisions. Indeed, reports suggest that dysregulation of microRNA expression in HSCs can lead to AML development.

Rationale: We and others have reported a significant down-regulation of microRNA 199b (miR-199b) in bone marrow from AML patients. Recently, miRNA expression analyses revealed that miR-199b is markedly enriched in hematopoietic stem cells (HSC) compartment, suggesting that miR-199b may regulate HSC function. Our flow cytometry analyses of bone marrow cells showed elevated frequencies of primitive HSCs and myeloid progenitors in mice harboring genetic deletion of miR-199b.

Hypothesis: Loss of miR-199b affects proliferation of HSCs and myeloid progenitors, and favors myeloid differentiation.

Aim: HSC behavior is regulated by its microenvironment, thus the goal of this study was to assess whether the response of HSPCs lacking miR-199b to GM and GEMM depends on the tissue microenvironment in which hematopoiesis occurs.

Experimental Approach

In vitro. In this study, we used a colony-forming unit (CFU) assay as validated in vitro that can detect an increase or decrease in the frequency of hematopoietic stem and progenitor cells (HSPCs) proliferation and/or changes in differentiation potential in response to stimulatory agents, such as GM and GEMM. Therefore, CFU on GM and GEMM media was assessed to determine proliferation abilities of HSPC from miR-199b KO bone marrow and spleen after primary, secondary, and tertiary plating as compared to WT.

In vivo: A bone marrow transplantation study was utilized to assess the ability of miR-199b KO HSCs to repopulate blood cells in healthy host mice exposed to sublethal irradiation.

Results: We found that miR-199b deletion decreases HSCs clonogenic potential, but favors GM myeloid differentiation and propagation of myeloid-committed progenitors. CFU-GM and CFU-GEMM were decreased in the splenic HSPCs from miR-199b KO mice as compared to WT counterparts. These results showed that splenic hematopoiesis is altered in miR-199b KO mice. CFU-GM assays revealed a similar behavior of miR-199b-null HSPC from both the bone marrow and spleen niches, indicating that extracellular factors did not play a significant role in such differences. Our transplantation study showed that miR-199b KO HSCs have a greater ability to repopulate myeloid and lymphoid cells than WT HSCs.

Further exploration into the effect of miR-199b in HSC self-renewal and progenitor differentiation will help us to understand the functional role of miR-199b in early hematopoiesis.

Methods

Figure 1. Experimental design for in vivo BMT transplantation study. Bone marrow from WT and KO mice was harvested and transplanted to irradiated mice carrying a C4-D2 marker. Blood samples were taken from both mice for flow cytometry to determine donor chimerism (%). Donor chimerism was measured at 4, 8, and 12 weeks post-transplantation of BM from WT and miR-199b KO mice into sublethally irradiated host mice.

Figure 2. Experimental design for in vitro CFU assays. Bone marrow and spleen cells were harvested from WT and miR-199b KO mice. Cells were plated in Methocult GM or GEMM media for 7 days and progenitors were assessed for multiple CFU types. Cells were harvested at 12 days after plating and stained with antibodies to detect CFU.

Figure 3. miR-199b KO HSPCs from bone marrow (BM) have decreased colony-forming abilities on GM medium at first plating. A) Representative images of colony forming assays for HSPCs differentiation into myeloid progenitors (CFU-GM/GEMM) performed in BM cells from WT and miR-199b KO mice (n=3) for 2.0 x 10^3 lineage-negative cells. B) Representative images of colony forming assays of miR-199b null HSPCs on CFU-GEMM directing cells to multilineage differentiation (CFU-GEMM). Assays were performed in BM cells from WT and miR-199b KO mice (n=3) for 2.5 x 10^5 cells. C) Total CFU-GM and CFU-GEMM for BM cells in WT and miR-199b KO mice.

Discussion and Conclusions

In conclusion, our results indicate that attenuation of miR-199b activity promotes myeloid progenitor proliferation. We found that miR-199b deletion favors myeloid lineage commitment and expansion of myeloid progenitors when HSPCs were isolated either from BM or spleen, suggesting that these HSPCs features result from intrinsic changes rather than niche alterations. Reduced colony forming abilities of splenic HSPCs in miR-199b KO mice suggest regulatory effects of miR-199b on splenic hematopoiesis. Future studies will be focused to understand the mechanism of miR-199b regulation of myeloid progenitor lineage commitment.

Acknowledgments

This research was supported by generous donation of Mr. Hosten and Mr. and Mrs. Benoit established in the Thomas W. Holden & John and Holly Benoit Endowed Fund for Research Education. Scientific expertise was provided by Edward Jachimowicz and the MMCI Core facilities in Flow Cytometry and Progenitor Cell Analysis: Physiology; Molecular Phenotyping. I would also personally like to extend my deepest gratitude to Dr. Karaczyn, Dr. Sathyanarayana, and Jane Friedman for providing me with such a positive summer experience.
Modeling Clear Cell Renal Cell Carcinoma: Characterization of the Tumor Extracellular Matrix

Anna Deck, Kyle Bond, Peter Brooks, Leif Oxburgh
Maine Medical Center Research Institute

Abstract

Renal cell carcinoma (RCC) is the most common kidney cancer, affecting nearly 64,000 new patients every year. Clear cell renal cell carcinoma (ccRCC) is the most common form of RCC, yet few treatment options exist for patients with this disease. Due to tumor heterogeneity and patient differences, developing personalized treatments is a high priority for treating affected patients. A major challenge in developing patient-specific models is the complexity of the tumor microenvironment (TME). One important component of the TME is the extracellular matrix (ECM), which has not been well characterized in ccRCC. Here we sought to understand the composition and structure of ECM in individual patients. Tumor and matching normal ECM and cells were isolated from patient biopsies and characterized through LCMS mass spectrometry and immunofluorescence. Independent data acquisition and analysis using SWATH identified drastic changes in collagen content between tumor and normal cells. It was further determined that a denatured collagen environment increased cell adhesion and Akt signaling using an in vitro cell adhesion assay with the 786-O ccRCC cell line. XL313, a truncated RGD/AGE collagen epitope, was shown to be secreted by 786-O cells. Immunohistochemical staining of patient tumors showed aberrant expression of XL313 throughout specific ccRCC tumors. These findings suggest that we have designed a cell isolation method that is viable to be used to create a ccRCC model to study cancer-ECM interactions. Additionally, the findings show a potential role of denatured collagen in ccRCC growth.

Introduction

- Developing a ccRCC model requires isolation of ccRCC cells; cells were isolated from tumors, grown in culture, and depleted of fibroblasts.
- Three possibilities exist regarding how ECM changes; new ECM deposited by tumors, ECM is degraded, or ECM is remodeled and exists in an alternate form. We used mass spectrometry and immunohistochemistry to explore these options.
- 786-Os, a ccRCC cell line, were grown on normal and denatured collagen IV to study which environment is more favorable for cancer growth. Secretion of collagen was also studied.
- Remodeling of ECM by damage or proteases can release previously hidden collagen epitopes that affect angiogenesis. In this study, targeted XL313, a collagen epitope linked to increased angiogenesis and inflammation in other cancer types and shown to be secreted by macrophages.

Effects of remodeling on cryptic epitopes

- Cell isolation and characterization plan:
- Mass spectrometry: Tumor and normal tissues depleted of cells run through data independent acquisition LCMS mass spectrometry to characterize proteins of the ECM, and analyzed via SWATH.
- Differential adhesion: 786-Os were seeded onto collagen IV and denatured collagen IV. Optical density was used to measure mean cell adhesion and western blot of downstream collagen signaling molecules were run. 786-O conditioned media was collected and analyzed via western blot for secretion of collagen epitopes.
- Immunohistochemistry: Paraffin-embedded sections were stained with anti-XL313 and Collagen I, III, and IV following previously developed ECM staining procedure. H&E and Trichrome staining performed by MMCRI Histology Core.

Discussion

- Differential changes in ECM composition and structure exists between normal and tumor ccRCC. The relative abundance of collagens varies significantly between the two groups.
- 786-Os preferentially adhere to denatured collagen IV, which leads to increased downstream of integrin signaling.
- Secretion of denatured collagen XL313 by 786-Os suggests a novel role of XL313 in ECM remodeling in ccRCC.
- XL3313 collagen epitopes are highly expressed in tumors, but not in normal, indicating both intratumor and patient heterogeneity.

Results

- A) Summary of specific ccRCC patient diagnostics used for current study. B) Validation staining of ccRCC cell isolation. (Vimentin and PDGFβ) and costained with DAPI.
- Immunohistochemical staining for each of the following components were run. 786-O conditioned media was collected and analyzed via western blot for secretion of collagen epitopes. Western blot of downstream signaling molecules involved in αvβ3 integrin signaling pathway following seeding onto normal or denatured collagen IV. Raw, a macrophage cell line, was used as an XL313 positive control.
- A summary of specific ccRCC patient diagnostics used for current study. B) Validation staining of ccRCC cell isolation. (Vimentin and PDGFβ) and costained with DAPI.

Future Directions

- Differential adhesion onto denatured collagen repeated with isolated primary ccRCC cells.
- Confirm that XL313 is secreted by primary ccRCC cells, as well as other ccRCC cell lines.
- Scoring for adhesion and stiffness of ECM, as well as downstream signaling events, could be performed to validate adhesion assays.
- Xenograft 786-O cells into immune-compromised mice and treatment with XL313 antibody to understand the role of XL313 on tumor growth.

Acknowledgments

This project was funded by the National Institutes of Health. The authors wish to thank all technical support and staff at the immunohistochemistry core for their assistance. The authors wish to thank all technical support and staff at the immunohistochemistry core for their assistance. The authors wish to thank all technical support and staff at the immunohistochemistry core for their assistance.

Conclusions

- Method used to isolate cells from ccRCC tumors efficiently purifies cancer cells based on marker analysis. These cells would be appropriate for downstream disease modeling studies.
- Drastic changes in ECM composition and structure exists between normal and tumor ccRCC tissue. The relative abundance of collagens varies significantly between the two groups.
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Figure 1

- Tumor Grade Stage Gender Race Age
- Tp18-S108 2 pThs F White 60-69
- Tp18-S109 2 pThs M White 60-69
- Tp18-S114 3 pThs M White 80-89

Figure 2

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Figure 4

- Tp18-S108
- Tp18-S109
- Tp18-S114

Conclusions

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Incidence and Characteristics of Opioid-Related Cardiac Arrests at Maine Medical Center

Bailey West1,2, Teresa May3, John Dziodzio1, Tyler Nussinov1, Barbara McCrum1, Christine Lord3, Ashley Eldridge3, Deanna Williams1, Lee Lucas3, Philip Stone3, Richard R. Riker3, David B. Seder4

1Maine Medical Center Research Institute, Scarborough, ME; 2Harborview Medical Center, University of Washington, Seattle, WA; 3Department of Critical Care Services, Maine Medical Center, Portland, ME; 4Center for Outcomes Research, Portland, ME

Abstract

The incidence of Opioid-related Cardiac Arrests (ORCA) has increased at Maine Medical Center (MMC) from 2013 to 2018. In this study, we evaluated the incidence of opioid-related cardiac arrest (ORCA) at MMC, compared ORCA patients to non-ORCA cardiac arrest patients, and examined the differences in baseline characteristics, hospital course, and outcomes between ORCA and non-ORCA patients.

Introduction

Opioid-related cardiac arrest (ORCA)

Methods

A retrospective chart review was performed for patients admitted to MMC for cardiac arrest from January 2013 to July 2018. Each arrest was classified as ORCA or non-ORCA using a system based on the criteria described in [3]. Demographic, hospital course, and outcomes data collected from the International Cardiac Arrest Registry (iCARe).

Results

ORCA patients had worse functional outcomes than non-ORCA cardiac arrest patients. ORCA patients were significantly younger and healthier than other cardiac arrest patients but more likely to have withdrawal of life support due to severe brain injury. There was no significant difference in functional outcome and in-hospital mortality. ORCA patients were less likely to undergo coronary angiography and percutaneous coronary intervention.

Conclusions

The incidence of ORCA at MMC has increased from 2013 to 2017. ORCA patients were younger and more likely to have withdrawal of life support due severe brain injury compared to non-ORCA patients. There was no significant difference in functional outcome and in-hospital mortality.

Next Steps

Comparison of brain injury by sex and MBI between ORCA and non-ORCA patients

Characteristics ORCA patients geographically

Acknowledgements

This research was supported by the Data and Valley Risk-Adjusted Fund for Research Education and the Maine Medical Center Research Institute Summer Student Research Program.
Spry1 deficiency in mice shows fat depot specific alterations in adipose tissue responses to a high-fat Western Diet

Bridget Mellon1, Shivangi Pande2,3, Xuehui Yang1 and Robert Friesel2,3

1 Biology Department, Gordon College, 255 Grapvine Rd. Wenham, MA 01984
2 Graduate School of Biomedical Sciences and Engineering, University of Maine
3 Maine Medical Center Research Institute, 81 Research Dr, Scarborough, ME 04074

ABSTRACT

The aim of this project was to investigate the effects of Spry1 deficiency on adipose tissue responses to a Western Diet. Spry1 is highly expressed in the adipocytes and adipocyte progenitors. We looked for a specific "coronal structures" around adipocytes that are indicators of fibrosis. We observed decreased coronal structures in the epididymal adipose tissue of Spry1 -/- mice compared to Spry1+/+ mice. Our results also demonstrated that Spry1 -/- mice have a higher incidence of KLF4 and F4/80 positive cell staining compared with Spry1+/+ mice, indicating that the difference in fibrosis may be due to differential expression of the above markers. Thus, loss of Spry1 may be protective against Western Diet induced adipose tissue dysfunction.

RESULTS

- We observed decreased coronal-like structures in the adipose tissue of Spry1 -/- mice fed a WD when compared to Spry1+/+ mice. This suggests that Spry1 deficiency may alter the response of adipose tissue to hypercholesterolemia and this response leads to a less fibrotic appearance.
- There appeared to be an increase (in both intensity and quantity) of F4/80 and KLF4 positive cells in the adipose tissue of Spry1 -/- mice compared to Spry1+/+. This suggests an increase in macrophage infiltration of Spry1 -/- adipose tissue.
- Fibrosis is usually a result of macrophage accumulation (in this case, hypercholesterolemia-induced) and/or aging, and based upon our observed increase in F4/80 immunostaining we expected fibrosis to be higher in Spry1 -/- mice fed a WD. The unexpected presence of less fibrotic adipose tissue in the Spry1 -/- mice (especially eWAT) suggests Spry1 deficiency disrupts the development of fibrosis under hypercholesterolemic conditions. Understanding the role of Spry1 in adipose tissue responses to a WD requires further study and may provide unique opportunities for the treatment obesity, diabetes and cardiovascular disease.
- It appears that Spry1 -/- mice, while they have increased atherosclerosis, also have more normal (healthier) looking adipose tissue when fed a Western Diet.

INTRODUCTION

Atherosclerosis, an inflammatory disease characterized by arterial wall thickening and plaque accumulation, is a major cause of death worldwide. It presents itself as an inflammatory response initiated by activation of the endothelial layer surrounding the vessel wall. It's phenotype transition of the smooth muscle cells, from a dormant, contractile state, to a proliferative and migratory, synthetic state.

Our lab has established that Spry1, a regulator of receptor tyrosine kinase (RTK) signaling, plays a critical role in the maintenance of the contractile VSMC phenotype by inhibiting their migration. Spry1 deficiency disrupts the development of fibrosis under hypercholesterolemic conditions. Understanding the role of Spry1 in adipose tissue responses to a WD requires further study and may provide unique opportunities for the treatment obesity, diabetes and cardiovascular disease.

MATERIALS and METHODS

**Spry1 deficiency in mice shows fat depot specific alterations in adipose tissue responses to a high-fat Western Diet**

**INTRODUCTION**

Atherosclerosis, an inflammatory disease characterized by arterial wall thickening

**RESULTS**

- We observed decreased coronal-like structures in the adipose tissue of Spry1 -/- mice fed a WD when compared to Spry1+/+ mice. This suggests that Spry1 deficiency may alter the response of adipose tissue to hypercholesterolemia and this response leads to a less fibrotic appearance.
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- It appears that Spry1 -/- mice, while they have increased atherosclerosis, also have more normal (healthier) looking adipose tissue when fed a Western Diet.

**MATERIALS and METHODS**

- Spry1 -/- and Spry1+/+ mice were fed a 45% fat diet for 6 weeks. AAV-PCSK9-D377Y was injected into the mice in order to reduce LDL-receptor expression in the liver, inhibiting cholesterol absorption from the blood and creating a hypercholesterolemic state.

**ACKNOWLEDGMENTS**

We would like to thank the MMCRI Animal Facility and the MMCRI Histology Core for their services (NIH grants P30 GM106391 (R. Friesel, PI, and P20 GM121301, L. Liaw, PI).

**REFERENCES**


Crafting a resource guide for curriculum building in EM POCUS Continuing Medical Education

Campbell Belisle Haley, Tufts University School of Medicine Maine Track Program

Abstract + Background

Ultrasonography (ultrasound) is a technology that uses high frequency sound waves to generate moving images of tissue. Point of care ultrasound (POCUS) is ultrasound brought to the patient and performed in real time. POCUS began in the 1990s as a tool used to improve diagnosis, procedures, and screening in multiple specialties. POCUS has become particularly important in emergency departments. This project explored the scope of POCUS training in emergency medicine (EM), compared ways that competency in POCUS is assessed, and used literature review to create a compilation of resources that aims to improve competency-based POCUS education of Maine EM physicians.

Objectives

1. Review guidelines from experts and professional organizations to determine core applications of EM POCUS
2. Identify pre-existing ultrasound assessment tools and review associated validity evidence
3. Develop a needs-assessment survey to determine the current use and training needs of ultrasound in Maine emergency departments

Components of a high-quality CME Curriculum

1. Clear educational objectives: What will your participants learn?
2. Integrated feedback mechanisms: How will you determine what they learned?
   - Determine “core” ED applications of POCUS
   - Review assessment tools available + their validity
   - Create a way to assess needs of physician population
3. Detailed instructional methods: How will you teach them this material?

Methods

- Manual search and MEDLINE search for ultrasound training recommendations
- MEDLINE search for previous ultrasound assessment tools
- Consultation with author of previous needs assessment tool to adapt for Maine

Results

3 Tiers of Core POCUS Applications were developed according to consensus expert opinion

<table>
<thead>
<tr>
<th>Tier 1 (30%): Emergency Imaging</th>
<th>Tier 2 (50%): Vascular Imaging</th>
<th>Tier 3 (20%): General Imaging</th>
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<td>FAST Scan, RUQ Scan</td>
<td>Abdominal aorta scan</td>
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3 published assessment tools were identified through literature review, 26 had evidence of validation studies

- 2 Surveys
- 2 Multiple-Choice Tests
- 20 OSCE/SDOT Checklists

26 published assessment tools were identified through literature review, 8 had evidence of validation studies

Conclusions

1. Objectives for a CME curriculum should be based on a) expert opinion of required applications for EM POCUS and b) assessment of training needs for a physician population
2. Many tools exist to assess skills in emergency POCUS, but few have been tested for validity
3. OSCE/SDOT checklists have the most validity evidence, but this assessment method requires presence of US experts

Acknowledgments

I would like to thank the Maine Medical Mutual Insurance Agency for funding this project, Shelly Chipman and the entire Simulation team for supporting me in this project, Dr. Christina Wilson and Dr. David McKenzie for their guidance on this project, and the Maine Medical Center Research Institute summer program for facilitating this research experience. I would like to dedicate this poster to Dr. Randy Darby, who inspired me to look deeper into how we measure quality and competency in medical education.
Time Course of Cognitive Change after Severe Hypoglycemia

Charlotte Benoit¹, Lori Brodsky², MN, FNP, Irwin Brodsky², MD, MPH
¹Maine Medical Center Research Institute, ²Maine Medical Partners Endocrinology and Diabetes Center

Abstract

Hypoglycemia, or episodes of low blood sugar, is particularly prevalent in people with type 1 diabetes. This is because taking too much exogenous insulin can lead to extremely low blood sugar levels. This study focuses on the cognitive effects of severe hypoglycemia, which requires the aid of another person, and particularly Grade 4HG, defined as episodes of low blood sugar that result in seizure or unconsciousness. It is well known that during and immediately after a severe hypoglycemic episode, brain functioning is slower and thinking can be ‘foggy’. This study addresses the cognitive effects of severe hypoglycemia in adolescents with type 1 diabetes over the span of a month, instead of just in the immediate aftermath of an episode. Participants ages 12-21 take a computerized test to measure memory, reaction time, and visual processing, in order to assess their general cognitive functioning. Data from a pilot study suggests that adolescents who experience an episode of Grade 4HG have impaired visual and verbal memory 1-2 weeks later. Data also suggests that visual and verbal memory recover by one month after the episode. Based on these preliminary findings, it seems that certain cognitive abilities continue to be impaired even after hypoglycemic symptoms are no longer noticeable. The study team continues to conduct research and acquire data from adolescents who have recently experienced severe hypoglycemia, as well as control subjects.

Introduction

Type 1 Diabetes

Healthy

Diabetic

Methods

The study team is testing adolescents ages 12-21 who have recently experienced an episode of Grade 4HG, adolescents who have type 3 diabetes but have not recently experienced severe hypoglycemia, as well as adolescents without diabetes. Each participant takes a 25 minute computerized test originally meant to assess concussions called the ImPACT test. The testing is conducted four times over the course of a month.

Hypothesis

The study team hypothesized that adolescents ages 12-21 would experience a temporary impairment in their visual and verbal memory 1-2 weeks after experiencing an episode of Grade 4HG.

Summary & Conclusion

- Visual memory is impaired in adolescents with type 1 diabetes 1-2 weeks after experiencing an episode of severe hypoglycemia.
- Verbal memory is impaired in adolescents with type 1 diabetes 1-2 weeks after experiencing an episode of severe hypoglycemia.
- The memory impairments are temporary and cognitive functioning normalizes by one month after an episode of severe hypoglycemia.

Acknowledgements

This work is generously supported by the NIH and Maine Medical Center Research Institute. Thank you Dr. Irwin Brodsky and Lori Brodsky for your initial and ongoing work on this study. Thank you for your interest in this poster!
A needs assessment was conducted to explore ways to improve the educational system at MTC. Findings from a literature review and key informant interviews with MTC clinicians and patients revealed many gaps in MTC patient education.

Using the SDM and TTM models as conceptual frameworks for redesigning the system, an alternative educational pathway to treatment was proposed. The educational class has been reformatted into an online, modular based program offered to patients as an alternative. At the same time, many transplant candidates lack thorough understanding of treatment options. Knowledge deficits impact appropriate care following transplantation.

Conclusions

- Needs assessment revealed many problems and corrective measures to improve patient education.
- Nationally, there is a huge effort to improve patient education, given the prominence of kidney disease and the long wait for transplants.
- Alternative Pathway for Education prototype will undergo future refinement but contains essential elements for improved education: limited length, prioritizing SSM and TTM models, chunking of information.
- Hand-outs are in the process of a health-literacy evaluation for better understandability.

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Thank you to the entire Transplant Team, both at the hospital and at clinic. Thank you to my mentor, Dr. Whiting, as well as Dr. Han and Jessica Begley from LRC. I am very grateful for all the patients who allowed me to shadow and interview them.

Elisabeth Lualdi, Dr. Paul Han, Jessica Begley, Dr. James Whiting

for the Maine Transplant Center

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9
A Comparison and Analysis of C2 Nerve Root Sacrifice Technique with Clinical Outcome

Emma C. England, Jeffrey E. Florman MD; Deborah A Cushing RN
Department of Neurosurgery, Maine Medical Center

Table 3. Results from delayed follow up patients

<table>
<thead>
<tr>
<th># of Patients</th>
<th># Nerves</th>
<th>Time Range</th>
<th>Occipital Pain</th>
<th>Narcotics for head pain?</th>
<th>Type of Narcotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early f/u Office Visits</td>
<td>35</td>
<td>66</td>
<td>1-7 months (mean=2.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Delayed Phone Survey</td>
<td>17</td>
<td>31</td>
<td>0.5-4 years (mean=2.2)</td>
<td>4 of 17</td>
<td>1 of 17</td>
</tr>
</tbody>
</table>

Figure 3. Demonstrates the portion of the head effected by the C2 nerve. "A Variation of Type III Odontoid Fracture As Isolated Jaw Pain" By M Walid et al.

Methods

- Literature was reviewed in attempts to compare the described sacrifice techniques (Table 1).
- Inclusion of trauma patients requiring C1-2 fusion and C2 nerve root sacrifice between 2007 and 2017
- All of the patients charts were reviewed with attention ON incidence, narcotic use and numbness dictated in the to post-operative office visits by the surgeon.
- The routine C2 sacrifice method was described in detail by the surgeon.
- Telephone interviews were conducted using the American Chronic Pain Association (ACPA) scale to assess quality of life, and the International Classification of Headache Disorder (ICHD-3) scale to assess headaches and neck pain.

Results

66 C2 nerve roots were divided at the mid-portion of the C1 lateral mass using bipolar electrocautery. There were no instances of vertebral artery injury, transfusions, or CSF leakage.

Discussion

- C2 nerve root sacrifice is often used to minimize blood loss, operating time, risk of injury to the vertebral artery, and increase visibility.
- Literature review reveals minimal but variable description for cutting the nerve.
- The C2 sacrifice technique in this series is most comparable to that described by Kang et al (2012).
- 0 of 35 patients have symptoms consistent with ON at 3 month f/u with the surgeon
- 4 of 17 patients admitted to head/neck pain consistent with ON during delayed phone f/u
- It proves difficult to correlate sacrifice technique to occurrence of ON

Learning Objectives

1. C2 nerve root sectioning technique is poorly understood
2. C2 nerve root sacrifice has excellent long term outcome
3. Transection technique may influence clinical outcome

Acknowledgements

Thank you to all patients who elected to participate in the survey. Thank you to Dr. Florman for allowing me to use the data from his patients and for the knowledge to complete the study. Also, thank you to Debbie Cushing for her guidance.

References

Characterization of a Novel Mouse Model with Adipocyte-specific Transgenic Expression of Mesoderm Specific Transcript

Gary R. Kersbergen, Rea Anunciado-Koza and Robert A. Koza

Abstract

Mesoderm-specific transcript (Mest) is expressed variably among genotypically homogeneous mice and highly induced in adipocytes when mice are fed an obesogenic diet. The correlation of elevated Mest expression to increased body weight and fat mass gain mediate Mes in a potential epigenetic regulator of diet-induced obesity. In order to understand the regulation and function of Mest further, a conditional transgenic model was created using CAG promoter which can be activated by tissue-specific expression if Cre-recombinase. Transgenic mice were placed on a high fat diet and the adipocytes and fat pads were harvested. Mest was isolated from these tissues and analyzed by qRT-PCR in order to confirm that the transgenic model was tissue specific. qRT-PCR analysis demonstrated that Mest expression was variably induced in adipocytes when mice were fed an obesogenic diet. The correlation of elevated Mest expression to increased body weight and fat mass gain mediate Mes in a potential epigenetic regulator of diet-induced obesity.

Introduction

Epigenetics & Obesity

Epigenetics is defined as the study of how changes in an organism’s physical or nutritional environment initiate changes in gene expression, but not the underlying DNA sequence. Changes are brought about through changes in the expression or behavior of genes that function as epigenetic regulators. In this study, we sought to understand changes in gene expression, but not the underlying DNA sequence. Changes are brought about through changes in the expression or behavior of genes that function as epigenetic regulators. In this study, we sought to understand changes in gene expression, but not the underlying DNA sequence. Changes are brought about through changes in the expression or behavior of genes that function as epigenetic regulators. In this study, we sought to understand changes in gene expression, but not the underlying DNA sequence. Changes are brought about through changes in the expression or behavior of genes that function as epigenetic regulators. 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In this study, we sought to understand changes in gene expression, but not the underlying DNA sequence. Changes are brought about through changes in the expression or behavior of genes that function as epigenetic regulators. In this study, we sought to understand changes in gene expression, but not the underlying DNA sequence.

Methods

Tissues from transgenic mice were harvested and analyzed for Mest expression by qRT-PCR. Gene expression was measured in arbitrary units (AU) and normalized to TBP. Some founder lines were found to be more effective at overexpressing Mes than others.

Summary/Conclusions

To better understand the function and regulation of Mes in adipose tissue expansion, we generated a conditional CAG-FGF-Mes transgenic mouse model that overexpresses Mes only in adipose tissues.

Results

Characterization of various founder lines for Mes expression in gonadal, inguinal, and brown (BAT) adipose tissues. Gene expression was measured in arbitrary units (AU) and normalized to TBP.

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Podocalyxin Promotes Activation of STAT3 Signaling Pathway in Emergency Granulopoiesis

Jahanara Freedman12, Aldona Karaczyñ1 and Pradeep Sathyanarayana1
1Center for Molecular Medicine, Maine Medical Research Institute, Scarborough, Maine 2Wellesley College, Wellesley, Massachusetts

Abstract

Background: Neutrophils serve a critical function in the innate immune system by maintaining a frontline defense against bacterial and fungal pathogens. New generations of neutrophils as a result of increased myeloid progenitor cell proliferation in bone marrow in response to severe infection is called emergency granulopoiesis. To maintain healthy neutrophil numbers, the process of granulopoiesis is tightly regulated. Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth receptor that is the primary stimulus for neutrophil production during emergency hematopoiesis. Janus kinase, signal transducer and activator of transcription (STAT) pathway components are principle intermediates in the G-CSF receptor signaling cascade. Jak2 is one of the key downstream kinases stimulated by G-CSF. Once phosphorylated, Jak2 activates STATs to be transported to the nucleus, a cascade necessary for accelerating neutrophil production. During G-CSF-driven emergency granulopoiesis, STAT3 is required to boost immature neutrophil numbers in bone marrow and to regulate acute neutrophil mobilization.

Rationale: The signal transduction pathways that regulate emergency granulopoiesis are of significant interest as G-CSF is used therapeutically to increase circulating neutrophil counts. However, the underlying mechanisms directing G-CSF-responsive myeloid progenitor expansion are poorly understood.

Podocalyxin is a transmembrane protein belonging to the CD34 family and is widely expressed in hematopoietic cells. Previously, our laboratory discovered that mice lacking Podocalyxin (Podxl) had significantly elevated peripheral blood neutrophils following G-CSF treatment. Therefore, the long-term goal of this project is to understand how Podxl regulates granulopoiesis and fundamental maturation of neutrophils in bone marrow. In this study, we investigated whether loss of Podxl affects Jak3 basic activity of STAT3 in myeloid progenitors, and Jak3 activity of STAT3 in the emergency granulopoiesis due to response to G-CSF administration.

Experimental Strategy: Mice, lacking the Podxl gene in their hematopoietic cells, were used in this study. Effects of Podxl deletion on myeloid progenitor (GMP) cell production during homeostasis and G-CSF-driven emergency response were assessed. Flow cytometry analyses were applied to investigate phosphorylated levels of STAT3 in GMPs from wildtype (WT) and Podxl conditional hematopoietic knock-out (KO) mice injected with PBS or G-CSF.

Results: We found that loss of Podxl reduces activity of STAT3 during emergency granulopoiesis and homeostasis. These results coincided with reduced number of the GMP population in both Podxl WT and KO mice injected with G-CSF and those injected with PBS, suggesting that Podxl deletion restricts GMPs differentiation.

Conclusions: Podxl promotes positive effects of STAT3 in the granulocytic lineage that directs myeloid progenitor proliferation.

Future Studies: We hope to investigate whether Jak3 loss of Podxl affects myeloid progenitor’s cell-cycle progression and maturation in response to G-CSF, and if Podxl regulates neutrophil trafficking during emergency granulopoiesis.

Methods

We used Podxl conditional knockout (KO) mice model generated using the floxCre technique to delete the expression of Podxl in hematopoietic lineages. Wt mice and Podxl KO mice were injected with 125 μg G-CSF twice in two days to induce emergency response in hematopoietic system. Initially, reduction of myeloid progenitors (GMPs) were tested in wildtype and wildtype G-CSF treated animals. Tested groups included wt/wt, wt/KO with G-CSF (WT), G-CSF (KO), and wt/KO (WT) with G-CSF (KO). In the next step, optimal concentration of antibodies to detect Podxl expression in mice was determined. Rat IgG1 was used for isotype control. The primary antibody was pre-incubated with rat IgG1 and labeled by biotinylated anti-rat IgG1 antibody. For detection of phosphorylated STAT3, rabbit polyclonal antibody against phospho-STAT3 (Tyr705) antibody was used. To confirm the expression of Podxl mRNA in bone marrow, reverse transcription polymerase chain reaction was used. In the next step, we determined total protein levels of Podxl in bone marrow using Western Blot analysis. Statistical significance was performed using the Student’s Two-Tailed T-test for Significance. **p<0.01 ***p<0.001.

Results

Figure 3: Loss of Podxl reduces frequency of GMPs during homeostasis and G-CSF-induced stress granulopoiesis. A) Representative flow cytometry gating strategy in flow cytometry analysis. B) Podxl KO mice were injected with saline (ctrl) or with G-CSF (10μg) for 3 days. C) Frequency of GMP population in both Podxl KO and wt mice treated either PBS or G-CSF. Statistical significance was performed using the Student’s Two-Tailed T-test for Significance. **p<0.01 ***p<0.001.

Discussion and Conclusions

We found that absence of Podxl in mice decreases the total number of neutrophils at the steady state and in G-CSF-induced response. This negative effect of Podxl deletion on STAT3 phosphorylation coincided with markedly reduced number of GMPs. We found no difference in the total level of STAT3 in G-CSF-treated WT mice compared to wildtype mice, however total levels of STAT3 were increased in Podxl KO in steady-state, suggesting an elevation of regulatory feedback. Studies have shown that in steady state, STAT3 senses a negative regulatory function by suppressing accumulation of neutrophils in blood. This activity is attributed to STAT3-dependent regulation of a feedback inhibition of G-CSF signaling. We recently found that Podxl deletion increases accumulation of the immature myeloid subpopulation with the associated drop in mature neutrophils in the bone marrow, and increased levels of neutrophils in blood. We found that Podxl-deficient GMPs showed suppressed G-CSF responsiveness-growth relative to wild-type cells, however the underlying cellular response such as enhanced rate of differentiation of GMPs remained unclear. To conclude, our findings suggest that Podxl promotes the activation of STAT3 in the granulocytic lineage that directs G-CSF-responsive myeloid progenitor proliferation. In the future, we hope to explore the effects of the loss of Podxl on myeloid progenitor’s self-renewal, proliferation and maturation in response to G-CSF. We also hope to investigate if Podxl regulates neutrophil trafficking during emergency granulopoiesis.

Acknowledgements

This research was supported by the SRRP program; thanks to Dr. Kozu, Dr. Liow and Liz Berger. Thank you Dr. Sathyanarayana and Dr. Karaczyn for your guidance and support. MMCRI flow cytometry Core was used in this project.

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Relationship Between Travel Distance to Cancer Care Center and Outcomes in Ovarian Cancer

Jonathan Emery, Lee Lucas PhD, Leslie Bradford MD

Aim
The purpose of this investigation is to determine whether there is an association between proximity of patients to a comprehensive cancer center and mortality in ovarian cancer patients.

Introduction
In 2015, there were 1.2 million women with ovarian cancer, which resulted in 161,100 deaths worldwide. Ovarian cancer is the seventh-most common cancer, and the eighth-most common cause of death from cancer. In Maine, 14,070 women are expected to die from this disease in 2018.

There is evidence that high volume facilities produce better outcomes for patients. This has led to an argument in favor of the regionalization of medical facilities. A possible drawback of this strategy is an increase in travel distance for patients. To begin weighing the pros and cons of regionalization it is worth asking: Does travel distance affect mortality?

Methods
Data from 2004 through 2015 were abstracted from the National Cancer Database (NCDB), a nationwide oncology registry with information on more than 70% of incident cancers in the United States. The study cohort included 165,674 patients and were limited to those with an invasive diagnosis, treated at a reporting facility, and not missing data for travel distance or stage.

Exclusion Criteria
National Cancer Database Ovarian cancer instances 2004-2015 (N=194,828)
- Non-invasive ovarian cancer (N=1,151)
- Invasive ovarian cancer (N=193,677)
- Patients treated in non-reporting facility (N=6,786)
- Patients treated in reporting facility (N=184,891)
- Patients missing stage (N=16,805)
- Patients missing travel distance (N=2,412)
Final Cohort (N=165,674)

Study Variables
- Travel Distance
  - CROWFLY, which is an estimation of the distance between the ZIP code of the patient’s residence and the facility. In this analysis, we split the travel distance into quartiles so that about 25% of the patients were within each distance range as follows:
  - Q1: < 5.2 miles
  - Q2: 5.2 – 11.9
  - Q3: 12 – 31.1
  - Q4: > 31.1

Covariates
- Age
- Race/Ethnicity
- Stage
- Comorbidity score
- Type of Cancer Center
- Income
- Education
- Insurance
- Facility volume

Baseline Characteristics of Cohort

Odds Ratios
Without Covariates
With Covariates
Q1 vs Q2
Q1 vs Q2
Q1 vs Q2

OUTCOME VARIABLE:
30 Day Mortality
NCDB includes a binary variable, which tells whether a patient has died 30 days after their diagnosis.

Results
Multivariable logistic regressions were performed to obtain odds ratios for each category distance compared to the first quartile (Q1) as reference. Before controlling for covariates we saw significantly lower odds of mortality with longer travel distances. Without covariates we found: Q2 Odds Ratio (OR), 0.884 [95% CI, 0.788 to 0.991], Q3 OR, 0.724 [95% CI, 0.643 to 0.816], Q4 OR, 0.891 [95% CI, 0.797 to 0.997].

With covariates, we lost some of these significant odds ratios: Q2 OR, 1.042 [95% CI, 0.925 to 1.175], Q3 OR, 0.881 [95% CI, 0.777 to 0.999], Q4 OR, 0.952 [95% CI, 0.841 to 1.077].

Conclusion
After controlling for covariates, we can say that, overall, travel distance does not have a statistically significant relationship with 30 day mortality among ovarian cancer patients. The next step, for a more in depth look at this question, would be to examine patients who travel further specifically to higher volume facilities in comparison to those who travel shorter distances to lower volume facilities and then to see if any effect is statistically and clinically significant.
Background
Powassan virus (Flaviviridae: Flavivirus) is a vector-borne disease that circulates in parts of North America and Russia in two serologically-indistinguishable lineages: lineage I (POWV) and lineage II (deer tick virus [DTV]). This study looked at DTV.

• Recent evidence suggests DTV may exhibit nidality, meaning it exists in small microhabitats (hotspots) consisting of just the right mixture of environmental factors for the virus to propagate.2
• Exists primarily in a zoonic cycle between Ixodes scapularis ticks (deer ticks) and small-medium mammals (e.g. the white-footed mouse, woodchucks, and skunks).3
• ~10% of POWV human infections result in deadly encephalitis and 50% result in long-term neurological sequelae.4
• In the United States there has been a threefold increase in ~10% of POWV human infections result in deadly encephalitis and 50% result in long-term neurological sequelae.4

Methods
Field Collection
Flagged for ticks in ten transects (5 different habitat types) in the Wells National Estuarine Research Reserve in Wells, ME. Each transect made up of ten consecutive 10x10m plots. 1. Forest with invasive shrub species in the understory 2. Forest with sparse, native shrub species in the Understory 3. Edge (forest-field intersection) 4. Shrub (little-to-no tree cover) 5. Field (grassland) 6. Edge (Forest-Field) 7. Sparse Understory 8. Forest with sparse, native shrub species in the Understory 9. Edge (Forest-Brush) 10. Edge (Forest-Tree Cover)

Laboratory Testing
• Ticks were ground in PBS solution and aliquots of these were tested by RT-PCR, amplifying the NS-5 gene (806bp).
• Individual ticks from positive pools were then tested using the same RT-PCR protocol and any positives were confirmed by genomic sequencing.
• To determine rate of co-infection, DTV-positive ticks were then tested for B. burgdorferi, A. phagocytophylum, B. microti, and B. miyamotoi by PCR assay.

Results
Out of 373 total ticks tested, 10 adults were positive for DTV, making the overall infection rate 2.7%. Eight of the positive ticks came from a deciduous forest habitat with invasive plant species, namely Japanese barberry, in the understory.

<table>
<thead>
<tr>
<th>Transect Number</th>
<th>Habitat Type</th>
<th>Number of Ticks</th>
<th>Number of DTV Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Brush</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Brush</td>
<td>20 (0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Forest with invasive Understory</td>
<td>106 (29.4)</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Forest with invasive Understory</td>
<td>113 (30.2)</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Field</td>
<td>6 (1.6)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Field</td>
<td>10 (2.7)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Forest/Edge</td>
<td>14 (3.8)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Forest/Edge</td>
<td>21 (5.6)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Forest with Native/ Sparse Understory</td>
<td>67 (18.3)</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Forest with Native/ Sparse Understory</td>
<td>11 (2.9)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>373</td>
<td>10</td>
</tr>
</tbody>
</table>

The majority of nymphal and adult ticks were collected from a deciduous forest-habitat with an understorey mostly composed of invasive species of plants.

Discussion
This study investigated Powassan virus nidality and co-infection within Ixodes scapularis ticks in Wells, ME by collecting 373 ticks and using PCR assays and genomic sequencing to test for DTV. Positive ticks were also tested for co-infection with B. burgdorferi, A. phagocytophylum, B. microti, and B. miyamotoi.

• This novel finding indicates possible nidality (focality) for DTV that corresponds with habitats known to be favorable for deer ticks (i.e. deciduous forest with invasive shrub species in the understorey). Therefore, invasive shrubs, like barberry, may play a role in forming DTV-favorable pathobiocenoses. However, differences in sample sizes between transects means we need to exercise caution when extrapolating this data.

• The habitat type in which the most ticks were collected, deciduous forest with invasive shrub understory, is consistent with previous research. This is thought to be because some invasive shrub species are resistant to deer browsing, act as good habitats for small mammals, and provide shade for ticks.

• Our reported 2.7% infection rate is within the 0.6-5% range expected from previous reports.5 The actual infection rate may be higher because this study primarily took place during peak nymphal season, and nymphs may have lower infection rates than adults (unpublished data).

• Also similar to previous reports, only lineage II (DTV) was found in I. scapularis ticks,2,6,7,8 The 70% positivity rate for DTV and B. burgdorferi (Lyme) co-infected ticks corresponded with reports on Lyme infection rates in Wells, ME (annual CDC report 2012).

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References
Characterization of the Proteolytic Processing of CTHRC1
Kimberly L. Drew1, Qiaozeng Wang MS1, Yong-Ri Jin PhD1, and Volkhard Lindner MD, PhD1
1Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, Maine

Introduction:
Collagen triple helix repeat-containing 1 (CTHRC1) is a hormone that is currently being researched in the biomedical field for its role as a matrix of different health interests, including, but not limited to cancer, heart disease, inflammation, and other factors that influence bone growth and aging.6 As a CTHRC1 is a versatile hormone, the proteolytic processing of this hormone, and where it is active and secreted, could be essential information to use to CTHRC1 to further the understanding of its role in these different medical interests. The proteolytic processing of CTHRC1 is not clear, and more studies specifically, whether or not the hormone needs to be activated and if it does how it is activated and secreted. CTHRC1 is expressed in neuroendocrine cells, which require regulated secretion to be activated and the test to further conclusions that CTHRC1 might start and regulated secretions as well. CTHRC1 has a terminal lysine, therefore the CTHRC1 processing pathway, which regulates the amount of CTHRC1 to be secreted could be important for understanding the biological properties of CTHRC1.17 To characterize this possible mechanism, the conditioned medium and cell lysate were immunostained to observe the enzyme function and the terminal lysine of CTHRC1. These cell lines were also immunostained for endogenous CPE, resulting in not further understand of the importance of CPE. These included: observation of cell line that expressed endogenous CPE (A120) and observation of the cell line that had not done past 250) to understand the ways CTHRC1 influences secretion in these cells.

Methods:
Transfection- The spe and plasmid were transfected with various stimuli using Flamingo Gene Essence transfection reagent (Bionutrient Co., Ltd.) cells were washed 0.5% BSA in Tris buffer and were suspended in 1% BSA. Cells were then transfected for 48 hours with mixing and in the media at the terminal (vivo). The presence of CPE was indicated if the expression of CTHRC1 was increased in NIH3T3 and EC cells, and no other bands were observed in the CL or CM.

Western blotting- Western blotting of transfected 293-T cells with four plasmid constructs hFL, hFLΔK, VVD, and hFLΔK with the myctags versus the plasmids that do not have the myctags. hFLΔK shows the presence of CTHRC1 in the CL and CM, with no clear increase with CPE. Note the shift between the CPE+ and CPE- samples.

Results:

1. 293-T cells did not secrete CTHRC1 efficiently, even with the addition of CPE.

2. In the cell lysate, the lower three bands is the non-glycosylated version, whereas the upper one is the glycosylated version.

3. In the A120 and CHO cell transfected with hFL and PHDRK, the presence of a higher band in the conditioned medium and PLD of HFL, than that of the cell lysate, this indicates a further post-translational modification that occurs outside of the cell with these plasmids.

4. Depending on the cell line, the monoclonal CTHRC1 antibodies that were used showed non-specific bands that were specific to that individual cell line.

5. In the A120 cell transfected Western Blot there was no secretion outside of the cell especially with the difficulties in reading the Western Blot due to the non-specific bands of a single site, which was unexpected. Assuming that there were no difficulties with the transfection that indicates that this is the only minimally active to be secreted.

6. Pondeau results under mass spectrometry determined that CHO cell CM had its terminal lysine, and so the result of CTHRC1 not having expressed endogenous CPE does not disprove the hypothesis.

7. Holden in the CHO transfection, the samples transfected with 2M/myc, show no shift between the CM and CL. This may be caused by a shift in the non-terminal lysine influencing the post-translational modifications that take place outside of the cell.

8. Along with this observation, the 2M/myc Western Blot that displays the presence of the CTHRC1. The CM in 293-T cells may indicate that the c-terminal lysine is not modified by the ability of 293-T cells to secrete CTHRC1 efficiently.

9. In the 293 cells when co-expression with CPE/PHDRK, the top band is slightly increased with CPE and CPE appears to be a timer, which can only be the catalyzing involved in this process. This observation does not have a conclusion aside from the possibility that the levels of the protein in the cell are increased, but will continue to be explored in future experiments.

10. Mass spectrometry will be necessary to know these observations in the future, as this will allow us to determine where these processing and whether or not it is likely that this is caused by CPE.

Acknowledgements:
The work in this paper was not possible without the support of the members of the Lindner Lab: Volkhard Lindner MD, PhD, Armie Mangoba, and Mayasah Al Hashimi and many of the faculty of MCRI with all of their support, expertise, and patience. The funding for this work was given to Volkhard Lindner, MD, and the American Heart Association. Thank you so much for this opportunity!

References:
10. Mass spectrometry will be necessary to finalize these studies.

Figure 1. Schematic of the KINQ processing pathway showing the CTHRC1 proteolytic sites on the lysine.

Figure 2. Western blotting of cell lysate and conditioned medium showing the detection of CTHRC1 in the NIH3T3 and EC cells. The probing of these blots with Vli55 and 13E9 as indicated shows that CTHRC1 has a c-terminal lysine, which has not been fully modified.

Figure 3. Transfected 293-T cells show detection of CTHRC1 in the cell lysate and does not secrete into the conditioned medium, which could be due to the presence of CTHRC1 in the cell lysate.

Figure 4. Transfected 293-T cells with indicated plasmid and CPE does not appear to affect the secretion of CTHRC1. The CM and CL shows a shift in the CTHRC1 samples that are with and without CPE, indicating a shift in the CTHRC1 samples that are with and without CPE.
Introduction

- The American College of Medical Genetics, American Association of Pediatrics, American Association of Psychiatry, and other medical governing bodies recommend that all children with ASD are referred for genetic testing.1
- Unfortunately, these clinical recommendations are not reflected in practice throughout the ASD population.1,3
- Children with ASD seeking care in inpatient psychiatric units could benefit greatly from genetic testing due to the complex nature of their diagnoses and behavior, which often include lower verbal levels, intellectual disability, self-injurious behavior and emotional dysregulation.1

Objectives

- To examine the prevalence and findings of genetic testing in an inpatient community of children diagnosed with ASD.
- To look for potential factors within demographics, medical history, family history and behavior that could influence access, referral and reporting of genetic testing.

Methods

- 936 participants with ASD confirmed by direct assessment using the Autism Diagnostic Observation Schedule (ADOS) were recruited from the Autism Inpatient Collection1, a study conducted in 6 child psychiatric units in the US specializing in treatment of ASD.
- 207 were excluded due to missing information or inconclusive data (N = 729).
- Parents filled out a Demographic and Medical Intake Form at admission which included questions regarding previous treatment, family history and other medical history.
- Parents also filled out an Aberrant Behavior Checklist - Community Form at admission, scoring their child on 5 subcategories of behaviors from 0 = never a problem to 3 = severe problem.
- Bivariate logistic regressions were conducted to examine the of parent reported genetic testing in hospitalized children with ASD.

Table 1: Demographic Characteristics of 729 Children with Autism Hospitalized in a Specialized Inpatient Unit

<table>
<thead>
<tr>
<th>Demographic</th>
<th>No Genetic Testing (n=415)</th>
<th>Genetic Testing (n=314)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years) (ASD)</td>
<td>15.32 (4.45)</td>
<td>12.4 (3.34)</td>
<td>P = 0.204</td>
</tr>
<tr>
<td>Sex (Male) (N%)</td>
<td>238 (57.73)</td>
<td>150 (46.43)</td>
<td>X2 = 8.36</td>
</tr>
<tr>
<td>ADOS Module (N%)</td>
<td></td>
<td></td>
<td>X2 = 2.30</td>
</tr>
<tr>
<td>1</td>
<td>141 (53.55)</td>
<td>125 (39.95)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>44 (44.44)</td>
<td>40 (40.00)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>31 (9.67)</td>
<td>59 (42.15)</td>
<td></td>
</tr>
<tr>
<td>Parental Education (N%)</td>
<td></td>
<td></td>
<td>X2 = 17.44</td>
</tr>
<tr>
<td>of Less than High School</td>
<td>36 (24.21)</td>
<td>36 (28.06)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100 (62.05)</td>
<td>181 (24.91)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>77 (25.96)</td>
<td>94 (25.96)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>39 (11.4)</td>
<td>28 (15.5)</td>
<td></td>
</tr>
<tr>
<td>Household Income</td>
<td></td>
<td></td>
<td>X2 = 1.22</td>
</tr>
<tr>
<td>Less than $30,000</td>
<td>108 (26.26)</td>
<td>192 (26.91)</td>
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</tr>
<tr>
<td>$30,000 - $100,000</td>
<td>136 (33.03)</td>
<td>211 (27.51)</td>
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</tr>
<tr>
<td>$100,000 - $250,000</td>
<td>77 (20.82)</td>
<td>94 (24.15)</td>
<td></td>
</tr>
<tr>
<td>More than $250,000</td>
<td>39 (11.4)</td>
<td>28 (15.5)</td>
<td></td>
</tr>
</tbody>
</table>

- Of the total child sample, 588 (80.6%) were male and the average age was 12 (SD = 3.3).
- 436 (54.3%) children were non- or minimally-verbal (determined by ADOS Module administered).
- There were statistically significant differences in gender, ADOS module, and household income between the two genetic testing groups.
- Those with genetic testing were more likely to be non- or minimally-verbal, and have a household income of more than $160,000.

Figure 1: The Rate of Genetic Testing Within an Inpatient Population.

- 43.07% of parents reported having genetic testing for their child prior to their admission.
- 25.6% received a significant result from this testing.
- A significant result consists of any disorder or mutation found regardless of correlation to ASD.

Results

- 69.66% of parents with a significant result reported that genetic testing resulted in some “other” finding and 15.7% reported a Fragile X diagnosis.4
- Of the other results, 9.68% were disorders known to have ASD etiology4 and 8.06% were considered ASD candidate genes5.

Figure 2: Breakdown of the most common parent reported genetic testing results.

- Families with a history of bipolar disorder (p = 0.006) were 74.1% more likely to report testing, while families with a history of a genetic condition (p = 0.00) were 78% less likely.

A

- Children with a history of bipolar disorder (p = 0.006) were 74.1% more likely to report testing, while families with a history of a genetic condition (p = 0.00) were 78% less likely.

B

- Higher ABC sub-scores in Lethargy (p = 0.003) indicated a lower likelihood of reporting genetic testing and higher scores in Stereotypy (p = 0.017) indicated a higher likelihood of testing.

Conclusions

- Children seeking care in an inpatient psychiatric facility received genetic testing at a higher rate than the general ASD community (<40%), although the diagnostic yield for these tests matched the national standards (10-40%).1,5
- Access to genetic testing and it’s findings could greatly influence the course of care in this population as it reveals important distinctions in disorders and ASD subtypes.
- These results suggest that family medical history, child behavior, and demographic characteristics predict likelihood of genetic testing. These predictors may represent pathways or barriers to access to referrals for genetic testing and follow through by parents.
- Further research is needed to understand the exact pathways by which some families are referred and receive genetic testing while others do not.

References


Acknowledgements

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Physician-patient Communication About Genomic Tumor Testing: Perceptions Of Oncology Providers

Alexandra McCown, Caitlin Gutheil, MS, Hayley Mandeville, MPH, Eric Anderson, Ph.D., Paul Han, MD, MA, MPH
Center for Outcomes Research and Evaluation (CORE) at Maine Medical Center Research Institute (MMCRI)

Abstract
Genomic tumor testing (GTT) is a potentially valuable new technology that can make cancer treatment more "precise," but there are substantial uncertainties about its clinical value and appropriate use. Oncology physicians need to counsel cancer patients about both the value and uncertainties about GTT, but optimal strategies remain to be determined. This study explored oncology providers' perspectives on the essential content elements of physician-patient discussions about GTT. 76 oncology providers who attended an annual meeting of the Jackson Laboratory's Maine Cancer Genomics Initiative (MCGI) were surveyed regarding their views about the key elements of GTT and goals of communication, which were consistent with the ideal of shared decision making (SDM). Study findings will be used to design patient education and physician training programs to promote SDM in GTT.

Methods
• In April 2018, 76 physicians and clinical staff attended an annual 2-day MCGI conference, convened by JAX to educate and update providers on the progress of the initiative.
• Conference participants were surveyed about their beliefs and attitudes regarding GTT.
• Surveys consisted of both multiple-choice and open-ended questions, designed to assess perceptions of the key goals and elements of physician-patient discussions of GTT.
• Multiple-choice question (Key goals)
  “What do you think are the three (3) most important goals when introducing GTT to a patient?” (respondents chose from option list)
• Open-ended question (Key elements)
  “Given what you know about GTT, how would you introduce it to a patient?”
• Frequencies of multiple-choice responses were calculated.
• Analysis of open-ended items was conducted using qualitative methods.
  • Software-assisted coding with MAXQDA™

Key Content Elements of Discussions
“Given what you know about GTT, how would you introduce it to a patient?”

• Nature of GTT
• Uncertainty about GTT
• Potential Outcomes of GTT
• Uncertainty about Therapeutic Options
• Patient Expectations

Illustrative Quotations from Open-ended Responses

“Every cancer is unique / This is a way to utilize precision medicine and offer a more personalized treatment based on cancer genomics and identifying specific genes and mutations associated with patient’s individual cancer”

“Testing the cancer genome and not germline/ Looking for an unusual and novel alteration in the genome that may give us insight into additional ways to treat your cancer”

The chance of finding a practical – treatment is small but if found could lead to major benefit”

The results may help identify one, none, or several mutations in your tumor / we hope the results will help guide future treatment options, however we don’t always find mutations that we can take action on- or change the treatment”

Conclusions
• Cancer care providers identify several different goals for physician-patient discussions about GTT
  • The most commonly prioritized goals relate to informed and shared decision making, and managing patient expectations.
  • Providers identify a variety of different content elements for physician-patient discussions about GTT.
  • Key elements regarding uncertainty in GTT focus mainly on therapeutic options and incomplete evidence.
  • Future research directions: Replicate and assess the generalizability of the findings in a larger, more diverse sample.

References

Acknowledgments
Alexandra McCown was generously supported by the Konkel Family Endowed Fund for Research Scholarship. The Maine Cancer Genomics Initiative (MCGI) is funded by the Alfond Foundation, and conducted in partnership with the Jackson Laboratory and the MMCRI Center for Outcomes Research and Evaluation (CORE)

a.mccown207@gmail.com
Flow Cytometric Analysis of Circulating Microparticles After Cardiac Arrest

Nathan L. Pinnette¹, Mary Weatherbee¹, Joanne Dekay¹, M.S., Sarah Peterson¹, M.D., PhD, Angela Kosta¹, B.S., Haifeng Yin¹, PhD, Douglas Sawyer¹,², M.D., PhD, Michael Robich²,³, M.D., David Seder², MD, Sergey Ryzhov¹, PhD
¹Maine Medical Center Research Institute, Scarborough, ME
²Maine Medical Center, Portland, ME

INTRODUCTION
Cardiac arrest (CA) is an electrical malfunction of the heart that causes an irregular heartbeat (arrhythmia). Because of this arrhythmia, an insufficient amount of blood is pumped to the vital organs. CA has a survival rate of only 10 percent and those who survive suffer from post-cardiac arrest syndrome (PCAS). PCAS is defined as a condition after resuscitation following a massive ischemia-reperfusion injury to all organs most notably of but not limited to the brain. PCAS is characterized by development of systemic inflammatory response, which contributes to additional brain tissue damage.

Microparticles are tiny particles in our blood. The two major ways microparticles are created: 1) cellular activation/stress and 2) cell apoptosis. In addition, immune complexes add to the pool of circulating microparticles. Microparticles are known to cause inflammation, coagulation, and effect vascular function.

Since CA is associated with global ischemia/reperfusion-induced cellular stress and apoptosis, we hypothesized that the number of circulating microparticles should increase in CA patients.

METHODS
Study participants. Research was performed in accordance with study protocols approved by Maine Medical Center Institutional Review Board, which is accredited by the Association for the Accreditation of Human Research Protection Programs (AAHRPP). Post-CA subjects age 18 years or older, admitted to the ICU after a cardiopulmonary arrest and treated with Targeted Temperature Management were enrolled after informed consent of the medicolegal Power of Attorney. Subjects underwent phlebotomy at 6, 12, 24, 48, 72 and 168 hours after Return of Spontaneous Circulation (ROSC). Control subjects underwent Coronary Artery Bypass Graft (CABG) surgery. Inclusion criteria included patients 18 years of age or older scheduled for open heart surgery supported by cardiopulmonary bypass (CPB) at Maine Medical Center. Plasma Samples: Venous blood (10 ml) was collected from cardiac arrest and control CABG subjects using BD Vacutainer ACD tubes. Platelet-free plasma was prepared at room temperature using two-step centrifugation, each at 2,000 rpm for 20 minutes. After preparation plasma was stored at -80°C until further analysis.

Cerebral Performance Category (CPC): CPC is a neurological test based on a scale of 1 through 5. 1 being the best and 5 being the worst.
- 1: return to normal cerebral function and normal living
- 2: cerebral disability but sufficient function for independent activities of daily living
- 3: severe disability, limited cognition, inability to carry out independent existence
- 4: coma
- 5: brain death

Flow cytometric analysis: was performed using a MACSQuant Analyzer 10 (Miltenyi Biotec, Inc.) and the data were analyzed with WinList 5.0 software. TruCount and sigma microbeads were used to set gates and calculate the number of circulating microparticles.

Data: Data were analyzed with GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA). Comparisons between two groups were performed using two-tailed unpaired t tests. Comparisons between several treatment groups were performed using one-way ANOVA followed by Multiple comparison tests. A P value < .05 was considered significant.

RESULTS

Figure 1. Flow cytometric gating strategy to calculate the number of circulating microparticles in platelet-free plasma. Fluorescein isothiocyanate-labeled (FITC-labeled) microbeads are used to remove background noise and record the percentage of all events that occur in the gate. This diagram shows that all of the microbeads are removed by the 0.1 micrometer filter.

Figure 2. The number of circulating microparticles in CA subjects at 6 hours is significantly higher compared to control subjects (p = 0.003). Statistical significance was calculated using one-way ANOVA. P values from Durbin’s multiple comparisons test are indicated.

Figure 3. There is no difference in number of microparticles between CA and control subjects. Circulating microparticles were measured in platelet-free plasma of CABG subjects (n=36), survivors (n=19) and non-survivors (n=22) after CA at different time points after ROSC. Statistical significance was calculated using one-way ANOVA. P values from Durbin’s multiple comparisons test are indicated.

Figure 4. Good CPC scores have significantly higher numbers of circulating microparticles compared to undesired CPC scores for CA subjects 48 hours after ROSC. Circulating microparticles were measured in platelet-free plasma of good and poor CPC scores after CA at different time points after ROSC. Statistical significance was calculated using t tests. P values from Mann-Whitney test are indicated.

CONCLUSIONS
- The number of circulating microparticles is not different between CA and control (CABG) patients.
- The number of circulating microparticles is characterized by high variability on day two after cardiac arrest with significantly increased number of microparticles in CA patients with good CPC scores compared to CA patients with poor CPC scores.
- Immunophenotypical analysis should be performed to determine if CA induces changes in origin of circulating microparticles.
Sympathetic and sensory innervation of bone modulates remodeling

Nick Banks, Audrie Langlais BS; Audrey Bergeron MS; Roni Kunst MS; Adriana Leilis Carvalho (PhD), K. Motyl, (PhD)
Center for Molecular Medicine (CMM), Maine Medical Center Research Institute (MMCRI), Scarborough, ME, US.

Abstract

Bone remodeling is a continuous, ubiquitous process. Sympathetic nervous output - especially through neurotransmitter β-endorphin - shifts the equilibrium towards increased resorption and decreased bone formation. The myriad of factors by which the nervous system interacts with the skeleton (hormones, peptides, neurotransmitters, etc.) comprise a somewhat “black box” type system. There is observable input and output, but incomplete understanding of the internal workings. The use of immunofluorescent neural markers seems promising in shedding light on this system. However, immunohistochemistry (IHC) and confocal imaging of bone present difficulties. The requisite fixation time and decalcification reduces antigenicity. This diminishes signal strength, which in addition to the high auto fluorescence and low neuron density of bone has lead to few labs reliably able to perform these techniques routinely.

An efficient, reproducible technique for visualizing and quantifying the differential innervation of bones will be quintessential in understanding the complex network between bone and brain.

Methods and Materials

• Fixed, frozen tissue sections of long bones and L5 vertebrae from transgenic mice expressing GFP driven by sequence under trpm8 promoter.
• IHC, performed to visualize neural markers and trpm8 in fluorescent markers.
• Quantum dots (Figure 4) conjugated to secondary antibodies (Figure 3) in lieu of organic fluorophores, for signal amplification to compensate for high auto fluorescence and as proof of principle for future studies.
• Images (Figures 6,7) from confocal microscope analyzed with Autoquant and Imaris software.

Introduction

Bone remodeling is a continuous, ubiquitous process. Sympathetic nervous output shifts the equilibrium towards increased resorption and decreased bone formation. The myriad of factors by which the nervous system interacts with the skeleton (hormones, peptides, neurotransmitters, etc.) comprise a somewhat “black box” type system. There is observable input and output, but incomplete understanding of the internal workings. The use of immunofluorescent neural markers seems promising in shedding light on this system. However, immunohistochemistry (IHC) and confocal imaging of bone present difficulties. The requisite fixation time and decalcification reduces antigenicity. This diminishes signal strength, which in addition to the high auto fluorescence and low neuron density of bone has lead to few labs reliably able to perform these techniques routinely.

An efficient, reproducible technique for visualizing and quantifying the differential innervation of bones will be quintessential in understanding the complex network between bone and brain.

Results

Figure 2. Afferent sensory nerves relay local mechanical conditions to the brain

Figure 4. Quantum Dot Anatomy

Figure 6. Strong evidence supporting sensory innervation along periosteum of femur. Tropomyosin receptor kinase A (TrkA) is a receptor and localizes sensory nerve fibers. Confocal image with 20x objective, 405 nm excitation.

Figure 7. Inconclusive staining for sympathetic innervation in growth plate of Tibia/Fibula. Tyrosine hydroxylase (TH) is an enzyme involved in the generation and maintenance of dopaminergic and noradrenergic neurons. TH can be stimulated by various stimuli. The production of catecholamines and localizes sympathetic nerve fibers. Maximum intensity projection of confocal 10 μm stacks, 20x objective.

Discussion

Unfortunately, we have not yet optimized technique enough to isolate meaningful signal from background, although positive staining has generally been in the reasonable location but lacks any “nerve like” morphological characteristics. Must titrate to detergent concentration, antibody type/dilution/ incubation times to optimize staining. Rigorous negative controls and positive controls (both dorsal root ganglia) will be required to confirm specificity of assay results. Further investigation of trpm8 localization in bone needed to reveal any potential role in mediating bone homeostasis.

Future Directions

• Multiplex imaging (Figure 8) of multiple neural markers and receptor (TH, CGRP, TRPM8) to show co-localization, give clues to communication mechanisms.
• Quantitative comparison of neural organization and density between long bones and vertebrae.
• Study changes in neural density from neuropathy in mouse BTBR Ob/Ob diabetes model.
Differential Gene Expression in Adipose Tissue of *M. musculus* Fed a High Fat Diet

Samantha White, Larisa Ryzhova Ph.D., Josh Boucher Ph.D., Cal Vary Ph.D., and Lucy Liaw Ph.D.

Perivascular adipose tissue (PVAT) surrounds the systemic vasculature of the body where it acts as mechanical support and secretes cytokines that affect the profile of the underlying vessel. The direct proximity of PVAT to the adventitia of blood vessels and its secretion of vasoactive factors that regulate vascular tone make it especially relevant to the study of cardiovascular disease. In healthy individuals, PVAT produces anti-inflammatory and anticontractile cytokines that promote vascular health. In cardiovascular disease and obesity, dysfunctional PVAT reduces vasculization and increases inflammatory response and vasoconstriction. Currently, there are no known molecular markers unique to PVAT, which limits investigation into possible treatments to encourage a healthy PVAT profile. The discovery of PVAT specific markers would allow for the development of novel tools to study this depot, and could lead to clinically relevant interventions to improve vascular function.

**Proteins enriched in *M. musculus* adipose tissues on HFD**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>iWAT CD</th>
<th>iWAT HFD</th>
<th>BAT CD</th>
<th>BAT HFD</th>
<th>PVAT CD</th>
<th>PVAT HFD</th>
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<tbody>
<tr>
<td>S27A2</td>
<td>Very long-chain acyl-CoA synthetase</td>
<td>1,042</td>
<td>6,294</td>
<td>19,618</td>
<td>33,436</td>
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<td>16,485</td>
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<tr>
<td>HMCS2</td>
<td>Hydroxymethylglutaryl-CoA synthase</td>
<td>6,773</td>
<td>50,774</td>
<td>2,399</td>
<td>6,749</td>
<td>2,115</td>
<td>11,594</td>
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<tr>
<td>PN2</td>
<td>Perilipin-2</td>
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<td>219,927</td>
<td>19,137</td>
<td>33,803</td>
<td>14,437</td>
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<td>NKG1A1</td>
<td>Neutrin A1</td>
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<td>138,524</td>
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<td>HSD12</td>
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<td>Transglin-2</td>
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<td>1,083,330</td>
<td>198,420</td>
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</table>

**Gene expression in PVAT**

- **qRT-PCR amplification and analysis of relative transcript abundance**
- **RNA isolation: Tissue lysis and affinity chromatography purification**
- **Mechanical homogenization of experimental tissues**
- **Stringent validation of primers in multiple tissues**
- **Mapping of genomic DNA and mRNA and primer design for candidate genes**
- **RNA isolation: Tissue lysis and affinity chromatography purification**
- **qRT-PCR amplification and analysis of relative transcript abundance**

**Table 1. Candidate proteins displaying differential abundance from protein mass spectrometry of adipose tissues.** Tissues were collected from mice fed a high fat diet vs. control diet for twelve weeks.

**Transcript abundance of candidate genes in BAT, IWAT, gWAT**

**Fig. 3. Differential RNA abundance in adipose depots on HFD.** qRT-PCR results show differential RNA abundance in adipose depots on HFD. Y-axis shows relative percent difference of HFD vs. CD as calculated using 2^-∆∆Ct. Ppia (Cyclophilin A) was used as the reference gene. qRT-PCR reactions contained 10ng of cDNA and 300nM primer concentration. Cycle: 95°C, 3 min; 95°C 15 seconds, 60°C 30 seconds x 40 cycles. n=3.

**Conclusion and future directions**

The transcriptome did not directly corroborate the proteomic data, with the exception of Anxa1 and Tagl2. Moving forward, this experiment will be extended to include PVAT from CD and HFD mice, and the data from all adipose depots will be validated at the protein level via western blot. Candidate genes from the second category identified in the proteomic assay—proteins with higher expression in PVAT and BAT but no change or a decrease in gWAT—will be evaluated along the same course as outlined here. Genes determined to have differential expression in PVAT at the protein and transcript level, will be further investigated as potential PVAT markers.

**Acknowledgements**

I’d like to thank Dr. Lucy Liaw and the members of her lab, Larisa Ryzhova, Josh Boucher, Jessica Davis-Knowlton, Anne Harrington, Terry Henderson, Emily Cooper, & Jacqueline Turner for their assistance and support during this project. David Champlin (USM Biology Department Chair) for securing and facilitating funding for the USM fellowship. Cal Vary (MMCR Faculty Scientist III) for the proteomic analysis and Michele Karoška (MMCR Molecular Phenotyping Core Manager) for maintaining the 384 Thermocycler with which I have become intimately familiar.
Pediatric Interfacility Transfers – Association of Pre-transfer Vital Signs with Length of Stay at a Tertiary Care Center

Sarah Bunting BA, Leah Mallory MD, Logan Murray MD

Maine Medical Center, Department of Pediatrics, Portland Maine

Background

• Interfacility transfers are common in rural states where few hospitals admit children. Pediatric hospitalizations admitted via transfer cost $19.5 billion in 2012.1
• As many as 25% of transferred pediatric patients are discharged within 12 hours of arrival and do not have any further work-up.2
• Understanding what pre-transfer factors are associated with shorter LOS may help avoid unnecessary transfers.

Objectives

1) Determine whether an association exists between abnormality of pre-transfer vital signs and LOS.
2) Identify pre-transfer vital signs associated with morbidity, such as unexpected transfer within 24 hours from the inpatient units (IPUs) to the PICU.

Materials and Methods

• All pediatric direct admissions from referring hospitals and urgent care centers to the Maine Medical Center IPUs and PICU, as well as all transfers to the ED for evaluation by the pediatric hospitalist service during the months of August 2016-January 2017 were enrolled.
• The patient's electronic medical record and/or HealthInfoNet data were manually reviewed. Data were entered into a secure database (REDCap™).
• Medical Complexity was assigned using a standardized method.3
• Vital signs were determined to be abnormal or normal using the Pediatric Advanced Life Support (PALS) algorithm.4
• Statistical analyses were performed using SPSS™ statistical software, version 25 (IBM SPSS Inc, Armonk, NY).

Results

• Many patients were discharged quickly after transfer; 5.9% in <6 hours; 11.9% in <12 hours; and 30.9% in <24 hours.
• Most vital signs were reliably obtained (HR for 93.6% of patients; RR, 90.7%; temperature, 87.3%; spO2, 92.4%). BP was an exception at 47.9%.
• Patients with abnormal RRs before transfer have significantly longer LOS than patients with normal RRs (61 v. 38 hours, p= 0.007). The same finding was apparent for BP (57 v. 31 hours, p< 0.035).
• Abnormal HR, temperature, and spO2 alone did not correlate with LOS.
• When controlling for temperature, there was no significant difference in LOS for patients with pre-transfer abnormal HRs.
• Younger patients were less likely to have a recorded BP (p<0.001), with a 10.73 year gap in median ages.
• Apparent trend toward abnormal RR being associated with unexpected transfer to PICU in 24 hours. Six out of 8 unexpected transfers had abnormal RRs.
• Alternatively, median LOS increased with medical complexity (p< 0.028).

Conclusions

• This study demonstrates a significant association between both abnormal pre-transfer RR and BP with longer LOS in pediatric patients at a tertiary care facility.
• It supports no significant correlation between abnormal HR, temperature, and spO2 with LOS.
• These results may better help both referring and accepting providers predict the course of patient care after transfer.

Next Steps

• Further research is necessary to increase the generalizability of this study, with the addition of other hospitals.
• Create a guideline where accepting pediatric providers obtain all five vital signs before accepting the patient. This could allow for the study of another objective measure of decompensation such as Bedside Pediatric Early Warning System (BPEWS) with LOS.

Acknowledgments

Special thanks to Wendy Y. Craig, PhD at the Maine Medical Center Research Institute for her invaluable assistance with statistical analysis of data which was supported in part by the Northern New England Clinical and Translational Research grant U54GM115516.

Reference

3T3-L1 model of adipogenesis and effects of methionine restriction

Sharon Jordan, Emily Cooper, Lucy Liaw PhD

Maine Medical Center Research Institute, Scarborough, Maine.
Sharonjordan@smcme.edu

Abstract

Healthy adipose tissue has an important role as an endocrine organ that affects whole body health. One example is the secretion of adiponectin, a hormone that aids in regulating glucose levels and breakdown of fatty acid. There are several types of adipose tissue. Pervascular adipose tissue (PVAT) surrounds most of the large blood vessels including the aorta. PVAT has thermogenic and vaso-protective properties. In obesity PVAT exhibits structural and functional changes. Unhealthy adipose tissue can become inflamed and inhibit the beneficial adipokines. Methionine reduction has shown to increase health span in mouse models. The methionine restricted mice continued to maintain healthy weight and improved glucose metabolism. To understand how methionine restriction alters adipose tissues, we initially used the 3T3-L1 cell line. 3T3-L1 is a fibroblastic line that can be differentiated into adipocytes and will be a useful model for altering methionine during adipogenesis in vitro. We used an adipogenic cocktail to induce these cells along the path to mature adipocytes. Oil Red O staining was used to view lipid accumulation and we performed initial studies with different concentrations of methionine in the medium. Our next aim is to test if the D-isomer of methionine is metabolized differently than the L-isomer. This is important to develop an accurate optimal methionine concentration. Having a cell model will be beneficial for future study into activities of methionine restriction in adipocytes. In addition, it will set the stage for continued studies with primary cells derived from PVAT.

Materials and Methods

Culture and Differentiation 3T3-L1 model of adipogenesis

The 3T3-L1 cell line was used to study adipogenesis in vitro. The 3T3-L1 line has a fibroblast-like morphology when grown under standard culture conditions. (see fig.1) Media consists DMEM/F12 with 10% FBS. For differentiation, 3T3-L1 cells were grown to 75-80% confluence and switched to adipogenesis induction medium. After 72 hours induction cells were switched to maintenance medium. The maintenance medium was changed every three days, leaving 25% medium in the plate and adding 75% fresh maintenance medium. Cells are maintained in maintenance medium for 8 to 10 before collection.

Methionine Concentration Variations in Media

To make medium with varying concentrations of methionine, DMEM/F12 and DMEM high glucose with no methionine was ordered (Gibco). Dialyzed FBS was used to ensure no added methionine was present. Induction and maintenance medium was made using methionine the methionine free components. Methionine free medium is mixed with standard medium to obtain desired concentrations.

Analysis of Adipocyte Differentiation

To determine the level of lipid accumulation oil red o (ORO) staining was used. The ORO stock solution is 0.35g ORO per 100ml 100% isopropanol. The working solution is 3 parts ORO stock 2 parts diH2O (0.05µg/ml). Cells were fixed in 10% neutral buffered formalin. Formalin was added slowly to the side of the well and aspirated off after one hour. Cells were rinsed with diH2O. After aspirating off the diH2O, 60% isopropanol was added to the well and the plate rotated to cover the cells. The isopropanol was eluted with 100% isopropanol, and the absorbance of the solution was measured at 490 nm.

Results

3T3-L1 model of adipocyte differentiation

Effects of methionine modification on adipocyte cell number and differentiation

Conclusions and Discussion

We confirmed that 3T3-L1 cells have the capacity to differentiate into adipocytes based on oil red O staining of neutral lipids in the differentiated cells. Using these cells as a model to test the effects of methionine restriction on the adipogenic capacity of the cells, we found that cells containing a racemic mixture of 0.0184mM methionine (D and L isomers) had enhanced

Acknowledgements and sources of support

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Characterizing the pluripotency of human thoracic perivascular adipose tissue progenitor cells

S. Spencer Scott, Joshua Boucher, Xuehui Yang, Lucy Liaw

This work was possible thanks to the generosity of Beth DeTine and the Paul Gray Scholarship.

Abstract

While burgeoning research on perivascular adipose tissue begins to illuminate the complex relationship between these unique fat deposits and the blood vessels to which they are localized, broad cellular and molecular characterizations of PVAT are still sparse. Furthermore, work with PVAT specimens collected from human donors remains limited in this exciting area of vascular research. At the Liaw lab, thanks to a collaboration with surgery at Maine Medical Center, human thoracic PVAT samples have been collected incidentally during cardiac surgical procedures and primary human PVAT cells have been explanted from the collected tissue. This project attempts to explore the nature of these explanted cells, presumed to be the progenitor cells that reside among PVAT and replenish the tissue in periods of regeneration or growth. A major question regarding these cells is the extent of their pluripotency. To probe this question, this project attempted to induce the PVAT primary cells toward an adipogenic, osteogenic, and chondrogenic lineage in three concurrent induction assays. The cells were cultured for 14 days in their respective induction media and then fixed in formalin. Subsequently, the cells were stained using Oil Red O to assess the extent of their pluripotency. To probe this question, this project attempted to induce the PVAT primary cells toward an adipogenic lineage. The success of differentiation was assessed by staining for neutral lipid accumulation with Oil Red O as well as probing for expression of genes specific to mature adipocytes, such as PPAR and PLIN1 or perilipin 1. However, a major question still remains: How committed are these PVAT progenitor cells to an adipogenic lineage? This project seeks to begin to illuminate the pluripotency of these primary cells by examining their ability to differentiate toward other cell lineages, but incapable of induction toward an osteogenic lineage.

Background

Perivascular adipose tissue, or PVAT, is a specialized form of fat tissue that encompasses major vasculature in the body. While this tissue was once thought to be primarily structural in nature, PVAT is now understood to play a significant role in regulating vascular health. In metabolically healthy individuals, PVAT both promotes vasodilation and inhibits inflammation. In metabolically unhealthy individuals, as in conditions of obesity, PVAT expands through a process called hypertrophy and loses its protective functions, exacerbating the risk of cardiovascular disease. Since 2016, the Liaw lab at the MMCRI’s Center for Molecular Medicine has made the study of perivascular adipose tissue a primary focus of its work. Through a collaboration with cardiac surgery at Maine Medical Center, the Liaw lab has been able to receive human PVAT specimens collected incidentally during coronary artery bypass graft (CABG) procedures. To accommodate grafting the new vasculature to the aorta, the local PVAT tissue must be cleared away. These samples are then provided to MMCRI for multiple avenues of study, including exploration of primary cells.

Adipogenic

Fig. 1. Following 14 days of induction, the explanted human thoracic PVAT cells displayed a robust ability to differentiate toward an adipogenic lineage as assessed by the accumulation of neutral lipids, stained here with Oil Red O. In image 1c, the non-induced condition is shown at 14 days with virtually no apparent neutral lipid accumulation.

Osteogenic

Fig. 2. In the osteogenic induction assay human bone marrow mesenchymal stem cells were cultured alongside human thoracic PVAT cells, both in osteogenic differentiation medium. At the end of 14 days of induction, Alizarin Red was used to stain for free calcium and calcium compounds, evidence of mineralization and osteogenic induction. Staining of the bone marrow MSCs showed a markedly larger amount of mineralization than the PVAT cells, in which the staining was sparse. This suggests that the human thoracic PVAT cells were not able to differentiate toward an osteogenic lineage by the 14 day time point, unlike the MSCs.

Chondrogenic

Fig. 3. In the chondrogenic induction assay, shown in these images, human thoracic PVAT cells were cultured using a “micromass” technique which allows for the growth of a pellet of roughly 10⁴ cells in the center of a culture dish. These cells were cultured in chondrogenic differentiation media for 14 days and then the cell pellet was embedded in paraffin and stained using Masson’s trichrome, of which the blue stains for collagen. In multiple pellets of human thoracic PVAT cells,
Neoadjuvant Therapy versus Upfront Surgery for Resectable Pancreatic Cancer using the NCDB database: A Decision Analysis

William Olsen BA, Roberto Vidri MD, David Clark MD, Timothy Fitzgerald MD
Maine Medical Center, Department of Surgery, Portland Maine

Background

- Pancreatic adenocarcinoma (PAC) is the third leading cause of cancer-related death in the United States and an estimated 44,330 people will die from PAC in 2018.1
- The only curative treatment at this time is resection, yet only 10-20% of patients are considered clinically resectable at the time of presentation.2
- Currently there are no prospective clinical trials that have shown benefit of neoadjuvant therapy (chemotherapy first) compared to upfront surgery in resectable PAC patients, and there are only retrospective single-institution studies.3
- Studies have shown that receiving chemotherapy at some point is advantageous compared to just surgery, but the sequence is debated.4

Objectives

- Compare the overall survival rates of patients with stage I and II resectable pancreatic adenocarcinoma who receive neoadjuvant chemotherapy and surgery versus patients who receive upfront surgery and adjuvant chemotherapy.
- Use a decision analysis to compare neoadjuvant therapy and upfront surgery, including rates of dropout from each group.
- Identify therapeutic and pathologic characteristics of neoadjuvant and upfront surgery patients associated with improved survival.

Materials and Methods

- Retrospective cohort study utilizing the NCDB database from 2004-2015 comparing patients who received neoadjuvant therapy and surgery versus patients who received upfront surgery and adjuvant therapy.
- 32,498 patients were selected by the following characteristics:
  - Invasive behavior of the tumor
  - Histology of Carcinoma NOS (8010), Adenocarcinoma (8140), and Ductal carcinoma (8500)
  - TNM Stage I and II
  - T1N0M0, T1N1M0, T2N0M0, T2N1M0, T2N2M0
  - Patients undergoing palliative care were excluded
  - Patients who refused surgery were excluded
- Descriptive statistics were used analyze the data

References


Prospective, randomized controlled trials comparing neoadjuvant therapy against an upfront surgical approach are needed to better answer this question.
**Influence of Lysosomal Acid Lipase on Osteoblast Differentiation and Function**

**Talia Staiger¹, Elizabeth Rendina-Ruedy¹, Ron C. Helderman¹, Liv Palma¹, and Clifford J. Rosen¹**

¹Maine Medical Center Research Institute, Scarborough, ME, 04074

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**Introduction**

- Lysosomal acid lipase (LAL) is an enzyme in the lysosome that breaks down cholesteryl esters (CE) and triacylglycerides (TAGs).¹
- In humans, an LAL deficiency presents itself in two diseases:
  - Wolman Disease - infants experience severe hepatosplenomegaly and malabsorption and usually die before one year of age
  - Cholesterol ester storage disease - later onset and milder phenotype, resulting in CE storage in visceral tissues, hepatosplenomegaly, and hypercholesterolemia.¹
- LAL knockout (KO) mice have increased TAG and CE storage in various tissues, as well as defective brown adipose tissue, leading to hypothermia at room temperature.³
- Recently, our lab has demonstrated LAL KO mice also have decreased bone volume/total volume (BV/TV) in the distal femur metaphysis.

**Hypothesis and Expected Outcomes**

- We hypothesized that LAL supports osteoblast differentiation.
- It was expected that higher doses of Lalistat, an LAL inhibiting drug, would slow the differentiation of osteoblasts and result in less alkaline phosphatase (ALP) staining.

**Acknowledgements**

This internship was supported by the Summer Student Research Program at Maine Medical Center Research Institute. I would also like to acknowledge all of the members of the Rosen lab, Liz Berget, Drs. Lacy Linse and Rob Kona.

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**Methods**

**Bone Marrow Stromal (BMSC) Cell Culture**

- C57BL/6 mice were sacrificed and their femurs, tibiae, and iliac crests were obtained. Their bone epiphyses were cut off to remove the bone marrow through centrifugation with minimal medium. The bone marrow pellets were resuspended, distributed into flasks with 25 mL alpha MEM complete medium, and incubated for 48 hours.
- The media and non-adherent cells were aspirated off and the adherent cells (BMSCs) were treated with 3 mL of trypsin for three minutes before 20 mL alpha MEM complete medium was added. The cells were counted and plated at a concentration of 5.0 x 10⁵ cells per well in three 12-well plates.
- Harvest bone marrow
- Isolate BMSCs from total bone marrow
- Plate BMSCs

**Lalistat Treatment**

- After 48 hours, the alpha MEM complete medium was replaced with osteogenic medium containing 50 µg/mL ascorbic acid and 5 mM β-glycerophosphate. The cells received this differentiation medium for seven days.
- Throughout the differentiation, cells received various doses of Lalistat at either 0µM (Control), 25µM, 50µM, or 100µM. This treatment also lasted for seven days.
- At the end of the experiment (day 7) cells were stained for alkaline phosphatase (ALP), an osteoblast marker.

**Results**

**Figure 1. Alkaline Phosphatase Staining of BMSCs After 7 Day Lalistat Treatment**

- a) This plate, stained at 7 days, shows increased amounts of staining in the control group compared to the 100 µM group.
- b) This plate, stained at 7 days, shows slightly increased staining in the 100 µM group compared to control.
- c) This plate, with cells grown during a different week than the other plates, shows equal amounts of staining across the wells, and more staining compared to the first two plates at 7 days.

**Figure 2. 10X Images of BMSCs From Figure 1A on Day 5**

- a) A well from the 0 µM control group of bone marrow stromal cells
- b) A well from the 100 µM lalistat group of bone marrow stromal cells

**Conclusions & Summary**

- ALP stains showed mixed results amongst the three plates, making the results inconclusive even though the photographs showed less confluence in the Lalistat-treated wells.
- This project taught me the techniques of bone marrow harvesting, cell culture, osteoblast differentiation and ALP staining. Additionally, I was exposed to genotyping/PCR, Cre LoxP models, Seahorse XF96 technology, and Confocal Microscopy.

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**References**

(3) Duta-Mare, M. (2018). Lysosomal acid lipase regulates fatty acid channeling in brown adipose tissue to maintain thermogenesis. BBA - Molecular and Cell Biology of Lipids.
Predicting Neurological Outcomes Using Pupillometry During Targeted Temperature Management After A Cardiac Arrest

Tyler R. Nussinow, Mary E. Sawyer, Dave Seder MD, Philip Stone DO, Richard R. Riker MD
Maine Medical Center, Neurocritical Care, Maine Medical Center Research Institute

Abstract
A pupil’s reactivity to light during targeted temperature management (TTM) after a cardiac arrest can help predict the neurological outcome of a patient. This was investigated by examining pupillometry data from patients admitted to Maine Medical Center from August 2017 - July 2018. Neurological Pupil Index (NPI), percent constriction and pupil size were compared to previously-established thresholds, while this was the first study, to our knowledge, to establish a threshold for constriction velocity (via ROC curve). This data was conducted in order to find trends consistent with good/poor neurological outcomes in patients who suffer from cardiac arrests. A NeuroOptics NP-200 pupillometer was used by nurses every few hours (no specific time requirement) both during and after TTM in order to get objective pupil exams. Comparison of pupillometry data was done by using patient’s worst scoring eye (in each respective category) between Good or Poor outcomes and statistically analyzed by using Mann-Whitney, Chi-Squared, and Fisher Exact tests. This study supports that NPI (<0 or <3), percent constriction (<13%), and constriction velocity (<0.54mm/sec and <0.32 mm/sec) at different time periods (<6 hours after ROSC, <24 hours after ROSC, or ever) are good predictors in neurological outcomes of patients after a cardiac arrest.

Introduction

*A pupilometer is a device used to objectively measure pupil size and reactivity to light—a pupil’s reactivity is thought to be directly correlated with brain activity.1

*When a person has a cardiac arrest (CA), significant brain damage can result due to lack of oxygen to the brain. Targeted temperature management (TTM) has been demonstrated to reduce the amount of brain damage that occurs in these patients.1

*The goal of this study was to examine data from the pupillometer (specifically Neurological Pupil Index (NPI), percent constriction, constriction velocity, and pupil size) and compare the values to previously-established thresholds, while this is the first study, to our knowledge, to establish a threshold for constriction velocity (via ROC curve). This data was conducted in order to find trends consistent with good/poor neurological outcomes in patients who suffer from cardiac arrests. A NeuroOptics NP-200 pupillometer was used by nurses every few hours (no specific time requirement) both during and after TTM in order to get objective pupil exams. Comparison of pupillometry data was done by using patient’s worst scoring eye (in each respective category) between Good or Poor outcomes and statistically analyzed by using Mann-Whitney, Chi-Squared, and Fisher Exact tests. This study supports that NPI (=0 and/or <3), percent constriction (<13%), and constriction velocity (<0.54mm/sec and <0.32 mm/sec) at different time periods (<6 hours after ROSC, <24 hours after ROSC, or ever) are good predictors in neurological outcomes of patients after a cardiac arrest.

Methods

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Results


CPC 1: Good cerebral performance: conscious, alert, able to work, might have mild neurologic or psychologic deficit

CPC 5: Brain death: apnea, areflexia, EEG silence, etc.

Acknowledgements

Supported by a grant from Maine Medical Center Research Institute Summer Student Research Program

References


Conclusions

- Constriction velocity was an accurate predictor of poor neurological outcome.
- Constriction velocity <0.54mm/sec within 6 hours after ROSC associated with poor outcome.
- Constriction velocity <0.32 mm/sec 6+ hours after ROSC associated with poor outcome.
- NPI value <3 confirmed as an accurate predictor of poor neurological outcome.
- Percent constriction <13% confirmed as an accurate predictor of poor neurological outcome.
- Pupil size confirmed as not an accurate predictor of poor neurological outcome.

Next Steps

Compile data from previous years in order to more accurately be able to demonstrate pupillometry data significance in predicting patient outcomes.

CPC 5: Brain death: apnea, areflexia, EEG silence, etc.

ROC Curve CV at 6 Hours

ROC Curves NPI, CV, %C at 6 Hours

CPC 1: Good cerebral performance: conscious, alert, able to work, might have mild neurologic or psychologic deficit

CPC 5: Brain death: apnea, areflexia, EEG silence, etc.

GOOD

POOR
Electrical Cardioversion of Emergency Department Patients with Atrial Fibrillation

Victoria Vargas; Tania Strout, PhD, RN, MS; Andrew Perron, MD
Emergency Medicine Department, Maine Medical Center, Portland, ME

ABSTRACT
This is a retrospective review of electrical cardioversion cases performed in the emergency department over the last 6 years. By describing electrical cardioversion as a management option in preference to other treatments for atrial fibrillation, we will be able to identify factors that may predict success for electrical cardioversion as a primary management option. Most patients ultimately require electrical cardioversion despite attempts at conversion with medication. The average visit duration for cases of electrical cardioversion was 206.67 minutes, whereas chemical cardioversion requires hours of observation within the ED to prove success in converting to NSR. Of these patients requiring electrical cardioversion, 13.5% returned to the ED for atrial fibrillation or related event within 30 days.

INTRODUCTION
Atrial fibrillation (AF) is the most prevalent and frequently encountered heart arrhythmia in adults, especially within the emergency department (ED).

With frequent ED encounters of atrial fibrillation, medical costs, hospital visits and duration of procedures are high. Studies have assessed rate control with chemical cardioversion but unfortunately, successful conversion using medications takes hours of monitoring each patient within the ED with a lower success rate overall. Negative assumptions about complications from electrical cardioversion impede use at hospitals everywhere.

However, when used electrical cardioversion has the highest overall success rate for patients presenting with atrial fibrillation. Utilizing electrical cardioversion as a primary treatment method for atrial fibrillation will lead to faster patient turnarounds within the emergency department.

RESULTS

Emergency Department patients presenting with atrial fibrillation will experience shorter, less frequent visits when electrical cardioversion is utilized as a primary management option for atrial fibrillation.

CONCLUSIONS
• The average visit duration for patients requiring chemical cardioversion was 331.8 minutes, versus the average duration for those only undergoing electrical cardioversion was 206.67 minutes.
• Only 13.5% of patients returned to the ED within 30 days for atrial fibrillation or related event after undergoing electrical cardioversion.

REFERENCES

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Maine Medical Center, New Hampshire-INBRE, Dartmouth College ISURF Program
Characterization of exosomes secreted from human thermogenic adipocytes

Zainab Miguel, Chad Doucette, Aaron Brown
Maine Medical Center Research Institute, Scarborough, Maine, Southern Maine Community College, South Portland, Maine

Abstract
Accumulation of excess fat in white adipose tissue (WAT) is associated with an increase in risk for type 2 diabetes (T2D). Unlike WAT, activation of brown adipose tissue (BAT) burns calories by releasing energy stored in fats to generate heat. This process, termed thermogenesis, occurs positively with a reduced risk for T2D. In addition to its fat burning potential, secreted factors derived from activated BAT may enter the circulation and reduce diabetic symptoms such as insulin resistance in other tissues. The mechanisms by which these secreted factors act on distant tissues may in part be due to their transport inside extracellular vesicles, known as exosomes. Exosomes carry a diverse array of signaling molecules, including microRNAs, proteins and lipids that are transported and released into recipient cells and tissues, potentially through direct homing via specific cell surface receptors. The goal of this project was to determine if brown adipocytes grown in cell culture secrete exosomes that contain miRNAs that may harbor anti-diabetic properties. We found that miRNA-32, a miRNA previously shown to activate brown adipocytes in a cell autonomous manner, can be packaged into exosomes and secreted. These results suggest a hypothesis whereby BAT may export exosomes containing miRNA-32 that activate other adipocytes and increase energy expenditure, thereby reducing fat accumulation. Overall, administration of brown adipocyte-derived exosomes to patients may be therapeutically beneficial for controlling T2D if they can be harvested in sufficient quantities from cultured cells.

Hypothesis
We hypothesize that activated human brown adipocytes grown in cell culture can be used to isolate exosomes to determine miRNAs that may alter gene expression in an anti-diabetic manner in distant tissues. The major goal of this project was to develop the methods necessary to isolate exosomes and determine their miRNA content.

Methods
1) Grow brown adipocytes in culture.
2) Isolate exosomes from conditioned medium.
3) Purify microRNA from isolated exosomes.
4) Make qRNA from exosomal microRNA
5) Test expression of microRNAs previously shown to be present in exosomes secreted by brown adipocytes in vivo.

Results

Analysis of RNAs isolated from exosomes using the Agilent Bioanalyzer
RNA isolated from either brown adipocytes or their exosomes was analyzed with the Agilent Bioanalyzer. Exosomes isolated from brown adipocyte exosomes showed a gel band in the approximate range expected for miRNAs (25-200 base pairs)

Amplification of microRNAs from exosomes
miRNAs were extracted from exosomes isolated from brown adipocytes using the Qiagen miRNeasy Micro Kit. miRNAwere converted to cDNA using gene specific reverse transcription primers for 3 exosomal miRNA markers previously shown to be expressed by brown adipocytes, including miRNA-32a, miRNA-99b and U6 snRNA. U6 snRNA is a non-coding small nuclear RNA commonly used as an internal control to normalize miRNA expression in cells. cDNA was amplified by qPCR using TaqMan probe based assays for these 3 targets. These data suggest that we were able to successfully isolate microRNAs from the exosomes of brown adipocytes.

miRNA-32 is enriched in exosomes compared to brown adipocytes
miRNA-32 expression was previously shown to be necessary for activation of brown adipocytes (Cell Rep. 2017, 19(6):1229-1246). We found that this miRNA is enriched in exosomes (>85 fold) compared to brown adipocytes they were derived from. This suggests that miRNA-32 may be secreted by brown adipocytes and play a role in cellular communication, including the possibility of inducing the formation of brown adipocytes in white adipose tissue. This could then lead to an increase in energy expenditure and loss of body fat.

Summary
We have demonstrated that activated brown adipocytes grown in cell culture secrete exosomes, similar to what occurs naturally after cold induction in vivo. These exosomes express microRNAs known to be expressed in brown adipocytes, which may play a broader role in communicating with other adipose tissue depots. Thus, cell cultures of activated brown adipocytes may provide an easy method to determine novel microRNAs that regulate metabolism in distant tissues. Future studies will be directed at sequencing microRNAs from exosomes isolated from cell cultures of brown adipocytes in our search for molecules that may regulate type 2 diabetes.

Acknowledgements
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The Role of Lipid Metabolism in Multiple Myeloma

DeSchiffart, Abigail1; Masarwi, Majdi1; Reagan, Michaela R.1,2,3
1Maine Medical Center Research Institute, 2Tufts University School of Medicine, 3University of Maine Graduate School of Biomedical Science and Engineering

INTRODUCTION

Multiple Myeloma (MM) is the second most common type of hematological cancer, formed from a series of oncogenic mutations to the plasma cells of the bone marrow (1).

Initially patients respond well to chemotherapeutic treatment, but almost all eventually develop resistance to these treatments and experience relapse.

Myeloma thrives in the unique and complex bone marrow microenvironment. Also, within the bone marrow are bone marrow adipocytes (BMA) that form bone marrow adipose tissue and account for 50-70% of the total bone marrow volume. It is believed that BMAT provides a source of energy that aids in multiple myeloma cell metastasis (2).

Fatty acid oxidation is the process by which cells convert long chain fatty acids into NADH, FADH2, and ATP in the mitochondrial matrix. It is the first, yet rate limiting enzyme of the carnitine system and subsequently of fatty acid oxidation (3).

Etomoxir (Eto) is a pharmacological irreversible inhibitor of CPT1, effectively inhibiting fatty acid oxidation.

In other cancers, such as breast and prostate cancer, inhibiting fatty acid oxidation with the use of etomoxir has been proven to reduce cancer cell viability and proliferation.

Recently etomoxir has been shown to have off target effects by inhibiting complex one of the electron transport chain at high dosages (4).

In addition to a potential energy source, BMAT has been shown to increase MM’s resistance to chemotherapeutic treatments (5).

We are examining the effect of etomoxir on different MM cell lines and if it increases MM sensitivity to chemotherapeutic drugs.

Objective and Aims: An in vitro investigation at the effects of inhibiting CPT1 on MM cells and to design a drug combination treatment that effectively reduces MM cell viability.

Hypothesis: Inhibiting fatty acid oxidation in multiple myeloma cell lines will reduce cell viability and increase their sensitivity to other chemotherapeutic drugs.

RESULTS

1. In Vitro Measurement of Cell Viability using Bioluminescent Imaging for MM Cells Treated with Etomoxir

Cell viability of (A) OPM2 and (B) MM1R cells treated with etomoxir (eto) at 0 µM, 5 µM or 12.5 µM, in addition to various doses of bortezomib (bort), specifically 0 nM, 0.25 nM, 1 nM, or 5 nM. Bioluminescence was used to measure tumor cell number and was read 72 hours after the drugs were administered. OPM2 cells were seeded at 20,000 cells/well in 96-well plates. *, p<0.05; ***, p< 0.0001.

2. In vitro Combination Treatment of Etomoxir and Bortezomib

OPM2: Etomoxir + Bortezomib

MM1R: Etomoxir + Bortezomib

3. In vitro Combination Treatment of Etomoxir and Dexamethasone Co-Cultured with MSCs

MM15 cells were directly co-cultured with mouse mesenchymal stem cells (mMSCs) for 24 hours before etomoxir (5µM) and dexamethasone (0.5µM) were administered. The mMSCs were seeded at a cell density of 7500 cells/well. The MM15 cells were seeded at a cell density of 5000 cells/well.