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

Follow this and additional works at: https://knowledgeconnection.mainehealth.org/jmmc

Part of the Medicine and Health Sciences Commons

Recommended Citation
(2019) "Poster Presentations from the 2018 Maine Medical Center Research Institute (MMCRI) Summer Student Research Program," Journal of Maine Medical Center: Vol. 1 : Iss. 1 , Article 16.
Available at: https://knowledgeconnection.mainehealth.org/jmmc/vol1/iss1/16

This Supplement is brought to you for free and open access by Maine Medical Center Department of Medical Education. It has been accepted for inclusion in the Journal of Maine Medical Center by an authorized editor of the MaineHealth Knowledge Connection. For more information, please contact Dina McKelvy mckeld1@mmc.org.
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 oncogenic mutations to the plasma cells of the bone marrow (3). 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 (BMAs) that form bone marrow adipose tissue and account for 50-70% of the total bone marrow volume. It is believed that BMAFs provide 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, FADH, 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, 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 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).

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

1. In vitro Measurement of Cell Viability using Calcein/Fluorescein Imaging for MM Cells Treated with Etomoxir

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Drug Concentration</th>
<th>Drug Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM1S</td>
<td>Eto 0 µM</td>
<td>Bortezomib</td>
</tr>
<tr>
<td>MM1R</td>
<td>Eto 0 µM</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>OPM2</td>
<td>Eto 0 µM</td>
<td>Combination</td>
</tr>
</tbody>
</table>

2. In vitro Combination Treatment of Etomoxir and Bortezomib

A combination treatment was designed to target etomoxir with another anti-myeloma drug, bortezomib.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Drug Concentration</th>
<th>Drug Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM1S</td>
<td>Eto 0 µM</td>
<td>Bortezomib</td>
</tr>
<tr>
<td>MM1R</td>
<td>Eto 0 µM</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>OPM2</td>
<td>Eto 0 µM</td>
<td>Combination</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Drug Concentration</th>
<th>Drug Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM1S</td>
<td>Eto 0 µM</td>
<td>Bortezomib</td>
</tr>
<tr>
<td>MM1R</td>
<td>Eto 0 µM</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>OPM2</td>
<td>Eto 0 µM</td>
<td>Combination</td>
</tr>
</tbody>
</table>

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 rule out any claims of off target effects.

REFERENCES


Conflicts of Interest: There are no conflicts of interest in this work to disclose.
Loss of miR-199b promotes expansion of myeloid-committed progenitors differentiated from bone marrow and spleen HSCs

Aidan McGory¹, Aldona Karaczyń, Edward Jachimowicz¹, Pradeep Sathyanarayana¹
¹Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, Maine

Results

KO

CFU

Figure 1. Experimental design for in vitro colony-forming unit (CFU) assays. A) Representative images of colony forming assays for HSC/PNs differentiated on GM medium at first plating. Assays were performed for 2.0 x 10⁴ cells. B) Representative images of colony forming assays for HSC/PNs differentiated on GEMM medium at first plating. Assays were performed for 2.0 x 10⁴ cells.

Figure 6. miR-199b KO mice show increased splenic progenitor proliferation as compared to WT mice. A) Representative image of serial colony-replating assays of secondary plating for myeloid progenitors (CFU-GM) performed in spleen cells from WT and miR-199b KO mice (n=3) for 2.5 and 5.0 x 10⁴ cells. B) Representative image of serial colony-replating assays of secondary plating for granulocyte, monocyte, erythroid, myeloid and megakaryocytes progenitors (CFU-GEMM) performed in spleen cells from WT and miR-199b KO mice (n=3) for 2.5 and 5.0 x 10⁴ cells.

Discussion and Conclusions

In conclusion, our results indicate that attenuation of miR-199b activity favors myeloid lineage commitment and expansion of myeloid progenitors when HSC/PNs were isolated either from BM or spleen, suggesting that these HSC/PNs features result from intrinsic changes rather than niche alterations. Reduced colony forming abilities of splenic HSPC 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 MMCR 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 Jana Friedman for providing me with such a positive summer experience.
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 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 αvβ3 cell adhesion assay with the 786-O ccRCC cell line. XL313, a truncated RGDAGGE 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 prostates can release previously hidden collagen epitopes that affect angiogenesis. In this study we targeted XL313, a collagen epitope linked to increased angiogenesis and inflammation in other cancer types and shown to be secreted by macrophages.

**Methods**

- Cell isolation and characterization plan:

**Results**

- XL313 collagen epitopes are highly expressed in tumors Tp18-S108 and Tp18-S109, but not in Tp18-S114, indicating both intratumor and patient heterogeneity.

**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 exist between normal and tumor ccRCC tissue. 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.
- XL313 collagen epitopes are highly expressed in tumors Tp18-S108 and Tp18-S109, but not in Tp18-S114, indicating both intratumor and patient heterogeneity.

**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.
- Scanning for an alongside other TME component cells should be performed to validate ability for cells to interact with XL313 epitope.
- Treatment of cell lines with anti-XL313 and measuring effect using a collagen invasion assay.
- Xenograft 786-O cells into immune compromised mice and treatment with XL313 antibody to understand the role of XL313 in tumor growth or inv

Acknowledgements

This project was funded by the National Institutes of Health. Thank you to Mandy for help in doing and filming the video used in the immunofluorescence portion of the project. Thank you to Dr. Lisa Lurain at the University of Pittsburgh for providing ccRCC tissue and cell lines.

References

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

Bailey West1,2, Teresa May1, John Dziodzio1, Tyler Nussinow1, Barbara McCormick1, Christine Lord1, Ashley Eldridge1, Deanna Williams1, Lee Lucas1, Philip Stone1, Richard R. Riker1, David B. Seder1

1Maine Medical Center Research Institute, Scarborough, ME; 2Honors College, The University of Maine, Orono, ME

Abstract

- The incidence of ORCAs increased at MMC and statewide from 2013 to 2018
- ORCA patients have worse functional outcomes than non-ORCA cardiac arrest patients

Methods

- A retrospective chart review was performed on medical records of MMC cardiac arrest patients and in prospectively collected registry
- Figure 2. Method for classifying cardiac arrests as ORCA or non-ORCA

Hypotheses

- ORCA patients demonstrated worse outcomes than non-ORCA patients
- The incidence of ORCAs increased at MMC and statewide from 2013 to 2018

Results

- ORCAs at MMC increased from 15 in 2013-2014 to 73 in 2016-2017
- Of 497 patients, 51 (10.3%) had ORCA
- ORCA patients were significantly younger and healthier than their cardiac arrest patients but were more likely to have witnessed arrest
- There were significant differences in laboratory values and medications at presentation
- ORCA patients were less likely to undergo additional cardiac interventions

Conclusions

- The incidence of ORCAs at MMC has increased by 73% from 2013 to 2017
- ORCA patients were younger with fewer comorbid conditions
- ORCA patients were less likely to have VT/VF
- ORCA patients were likely to have more severe brain injury
- ORCA patients were less likely to undergo additional cardiac interventions

Acknowledgements

This research was supported by the Data and Vitals, R&R Willard Endowed Fund for Research Education and the Maine Medical Center Research Institute Summer Student Research Program.

References


Next Steps

- Compare patterns of brain injury by severity and MRI between ORCA and non-ORCA patients
- Characterize ORCA geographically throughout Maine
Spry1 deficiency in mice shows fat depot specific alterations in adipose tissue responses to a high-fat Western Diet

Brigid Mellon1, Shivangi Pande2,3, Xuehui Yang1 and Robert Friesel1,3

1 Biology Department, Gordon College, 255 Grapevine 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 with 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 crown-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.

MATERIALS and METHODS

- AAAS-PCK9-D377Y was injected into the mice in order to reduce LDL-receptor expression in the liver and creating a hypercholesterolemic state.
- Analysis of adipose tissue included immunohistochemistry with F4/80 and KLF4 antibodies, which are markers of macrophages and stem cells respectively.
- We looked for a specific “coronal structures” in fat depot specific alterations in adipose tissue responses to a high-fat Western Diet.
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

Ultrasoundography (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?
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

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

Acknowledgements
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.
**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* vs. *Diabetic*

A comparison of a non-diabetic pancreas and a type 1 diabetic pancreas. Adapted from Diabetes Daily. Causes of Type 1 Diabetes.

**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!
Introduction + Abstract

- Nationally, more than 115,000 patients are on the waiting list for a life-saving kidney transplant.
- At the Maine Transplant Center (MTC), 100 patients are on the waiting list, and about 50 transplants are performed in a given year.
- Patient Education at the MTC consists of a single educational class before evaluation.
- Educational class creates scheduling demands that delay evaluation & treatment.
- Many transplant candidates lack thorough understanding of treatment options.
- Knowledge deficits impact appropriate care following transplantation.

Many transplant candidates lack thorough understanding of treatment options. Patient Education at the MTC consists of a single educational class before evaluation. Educational class creates scheduling demands that delay evaluation & treatment. Many transplant candidates lack thorough understanding of treatment options. Knowledge deficits impact appropriate care following transplantation.

Objectives + Methods

- To improve the educational system for patients at the Maine Transplant Center, aimed at facilitating informed and shared decision making.
- To conduct a needs assessment, based on literature review and key informant interviews, guiding efforts to redesign the educational intervention and identify areas for improvement.

10 Weeks in a Nutshell

- Staff Interviews
- Presentation to Transplant Team
- Self-education on health literacy
- CDC scanning of content delivered during class (power point and handouts)
- Patients have low health literacy.

Conceptual Models

- SDM
- TTM
- SSM

- Patients have low health literacy.
- CDC scanning of content delivered during class (power point and handouts)

Module

1. Introductory
2. Understanding
3. Contemplation
4. Preparation
5. Action

- Patients who have completed Modules 1 and 2 have the chance to complete the Module series and forge a class of the Transplant Center.
- Patients complete the 3 remaining modules.
- Patients are able to access online education and corrective measures to improve patient education, given the prominence of patient education, given the prominence of

References + Acknowledgements

4. Waterman, AD et al. Educating Prospective Kidney Transplant Recipients to a Sun-Protection Education Program Delivered on Tablet Computers: Randomized Controlled Trial. 2015.
5. Robinson JK et al. Response Across the Health-Literacy Spectrum of Patients with End-Stage Renal Disease Pursuing Kidney Transplant. 2015.

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.

Table

<table>
<thead>
<tr>
<th>Module</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introductory</td>
<td>Understanding</td>
</tr>
<tr>
<td>Understanding</td>
<td>Contemplation</td>
</tr>
<tr>
<td>Contemplation</td>
<td>Preparation</td>
</tr>
<tr>
<td>Preparation</td>
<td>Action</td>
</tr>
</tbody>
</table>

Prototype: A 5 Part Online Power Point with Audio

- Alternative Pathway for Education
  - Limit the length.
  - Fit the format to the preferred way of learning.
  - Meet patients where they are in regard to treatment options and knowledge.
  - Explain complex issues in easy-to-understand language.
  - Lessen the amount of information presented at one time (chunking).
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

Abstract
The C2 nerve occupies a challenging anatomical position when inserting C1 lateral mass screws during atlantoaxial fixation and often requires root sacrifice or retraction. The method for C2 nerve sectioning is a potentially modifiable factor influencing clinical outcome and incidence of occipital neuralgia (ON). In this series, C2 nerve transection is performed routinely and clinical outcomes were assessed prospectively.

Introduction

Table 1. Review of C2 Nerve Root Transection Techniques

<table>
<thead>
<tr>
<th>Study &amp; Year</th>
<th>Technique</th>
<th>Sectioning Location</th>
<th>Patient Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aryan (2008)</td>
<td>Bipolar electrocautery (EC) with metzenbaum scissors</td>
<td>Proximal to the DRG</td>
<td>1 of 102 (p=0.010)</td>
</tr>
<tr>
<td>Squires and Molinari (2010)</td>
<td>Monopolar EC</td>
<td>Mid-portion of C2 articulation</td>
<td>0 of 14 (p=0.000)</td>
</tr>
<tr>
<td>Kang et al (2012)</td>
<td>Bipolar EC with Malis scissors</td>
<td>Mid-portion of C2 articulation</td>
<td>0 of 19 (p=0.000)</td>
</tr>
<tr>
<td>Yeon et al (2013)</td>
<td>Bipolar EC; bipolar EC &amp; knife or metzenbaum scissors</td>
<td>Proximal to the DRG or through the DRG</td>
<td>6 of 24 (p=0.250)</td>
</tr>
</tbody>
</table>

Table 1. An overview of literature describing C2 sectioning techniques and location for patients who underwent sacrifice of the C2 nerve.

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 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.

Table 2. Results from early office visits and delayed follow up

<table>
<thead>
<tr>
<th>Early f/u</th>
<th>Delayed f/u</th>
<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>Office</td>
<td>Phone</td>
<td>Early</td>
<td>35</td>
<td>66</td>
<td>1-7 months (mean=2.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Visits</td>
<td>Survey</td>
<td>Delayed</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: An Isolated Jaw Pain" By M Wald et al.

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) has been considered to promote obesity in mice fed a high fat diet. However, the role of Mest in determining obesity in response to a high fat diet has not been studied. To characterize the role of Mest in determining diet-induced obesity, we generated a conditional transgenic model in which Mest was overexpressed in adipocytes in a transgenic mouse backgound. Mest overexpression was confirmed using qRT-PCR and microarray analysis. The biological mechanism by which Mest functions as an epigenetic determinant of diet-induced obesity was further characterized.

Introduction

Epigenetics & Obesity

Epigenetics is defined as the study of heritable changes in an organism’s physical or environmental environment initiated by changes in gene expression without a change in DNA sequence. The understanding of epigenetics is important because it allows us to understand changes in gene expression that do not follow the standard DNA sequence. Understanding epigenetics can lead to the development of treatments for obesity and related diseases.

Methods

Transfection of HKD35 cells CAG-GFP-Mest and CAG-GFP-MestMyc (A,B) and co-transfection of cells with CAG-cre recombinase (C,D) resulted in reduced GFP signal for CAG-GFP vectors. Cells were quantified by qRT-PCR.

Results

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

Summary/Conclusions

To better understand the function and regulation of Mest in adipocyte tissue expansion, we generated a conditional CAG-GFP-Mest transgenic mouse model that overexpresses Mest only in adipocytes. The biological mechanism by which Mest functions as an epigenetic determinant of diet-induced obesity was further characterized.

Acknowledgements

University of Southern Maine – Maine Economic Improvement Fund (MEIF), Dr. Robert A. Koza and Dr. Rea Anunciado-Koza, Professor David Champlin, Summer Student Research Program (MMCRI), Liz Bergel and Dr. Lucy Liaw, Dr. Pradeep Sattarajankura and Dr. Abbas Karimi, MMCRI Transgenic core (Larisa Ryzhova, Lucy Liaw, Anne Harrington.)

Supported by pilot funding from P20GM121301 (COBRE; Lire)
Podocalyxin Promotes Activation of STAT3 Signaling Pathway in Emergency Granulopoiesis

Jahanara Freedman1,2, Aldona Karaczyn1 and Pradeep Sathyanarayana1

1Center for Molecular Medicine, Maine Medical Research Institute, Scarborough, Maine 2Wellesley College, Wellesley, Massachusetts

Abstract

Background: Neutrophils play 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 stimulator 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 and Jak3 are one of the key downstream kinases stimulated by G-CSF. Once phosphorylated, Jak2 activates STAT3 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 (Podcxl) had significantly elevated peripheral blood neutrophils following G-CSF treatment. Therefore, the long-term goal of this project is to understand how Podcxl regulates granulopoiesis and functional maturation of neutrophils in bone marrow. In this study, we investigated whether loss of Podcxl affects Jak3 basal activity of STAT3 in myeloid progenitors, and if Jak3 activity of STAT3 in the emergency granulopoiesis due to response to G-CSF administration.

Experimental Strategy: Mice, lacking the Podcxl gene in their hematopoietic cells, were used in this study. Effects of Podcxl 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 Podcxl conditional hematopoietic knock-out (ko) mice injected with PBS or G-CSF.

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

Conclusions: Podcxl promotes positive effects of STAT3 in the granulocytic lineage that directly affects myeloid progenitor proliferation.

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

Methods

We used Podcxl conditional knock-out (ko) mice model generated using the floxed Cre technique to ablate the expression of Podcxl in hematopoietic lineages. Wildtype and Podcxl cko mice were injected with 125 µg of G-CSF twice in two days to induce emergency response in hematopoietic system. Initially, isolation of myeloid progenitors (GMPs) were tested in wildtype and witdophybotic G-CSF deficient animals. Tested groups included wildtype(WT), WT+G-CSF, Podcxl-WT+G-CSF, Podcxl-ko with either G-CSF or PBS. BM cells were flushed with HOM medium supplemented with 5% FBS and antibiotics and centrifuged. BM cells were passed through 210 µm syringe and 0.45 micron strainer. 0.1% Bone Serum Albumin buffer and PBS 1x was added to the cells to be centrifuged. Cells were counted using hemocytometer. Red blood cells were removed by chemical lysis. Single cell suspension of BM cells was processed by EasySep Mouse Hematopoietic Progenitor Cell Isolation Kit according to manufacturer’s protocol (StemCell Technologies). Lineage negative population was obtained by immunomagnetic separation. Number of lineage negative cells is measured. The forward labeled with CD45+ and CD45+ and labeled by antibodies against CD44, CD31, CD135, CD45+ followed by fixation and permeabilization. Phospho-STAT3, STAT3, and isotype IgG antibodies were added separately to tested groups and incubated for one hour. Samples were submitted to flow cytometry for analysis. Flow cytometry data analysis was performed using Flowjo.

Figure 1: Flowchart diagram portraying methodologies of this study.

Figure 2: G-CSF induces GMP proliferation and STAT3 activation in vivo. Lineage negative c-kit positive, Sca-1 negative compartment containing myeloid progenitors from wildtype mice injected with either G-CSF or PBS in bone marrow. Number of GFP+GMPs were increased in Podcxl cko mice as compared to wildtype mice, however, total level of STAT3 were increased in Podcxl cko in steady-state, suggesting an essential role of Podcxl in normal hematopoiesis. These findings show that Podcxl deletion reduces the GMP population in vivo. Figure 3: Loss of Podcxl reduces frequency of GMPs during homeostasis and G-CSF-induced stress granulopoiesis. A) Representative gating panel showing expression of GMPs in bone marrow tissues from mice treated with PBS or G-CSF. B) Frequency of GMP population between Podcxl cko and wildtype mice treated with either PBS or G-CSF. Statistical significance was performed using the Student’s Two-Tailed T-test for Significance. *p<0.05 ***p<0.001.

Discussion and Conclusions

We found that absence of Podcxl in myeloid progenitor cells results in reduced level of STAT3 in G-CSF induced response. This negative effect of Podcxl deletion on STAT3 phosphorylation coincided with markedly reduced production of GMPs. We found no difference in the total level of STAT3 in G-CSF treated Podcxl cko as compared to wildtype mice, however, total level of STAT3 were increased in Podcxl cko in steady-state, suggesting an essential role of Podcxl in normal hematopoiesis. These findings show that Podcxl deletion reduces the GMP population in vivo.

We found that loss of Podcxl affects Jak3 activity of STAT3 in myeloid progenitor proliferation. Jak3 activity of STAT3 in the emergency granulopoiesis due to response to G-CSF administration. These results coincided with reduced number of the GMP population in both Podcxl ko mice injected with G-CSF and those injected with PBS, suggesting that Podcxl deletion restricts GMPs’ differentiation. These results coincide with the previous findings in our laboratory that mice lacking Podcxl had significantly elevated peripheral blood neutrophils following G-CSF treatment. Therefore, the long-term goal of this project is to understand how Podcxl regulates granulopoiesis and functional maturation of neutrophils in bone marrow. In this study, we investigated whether loss of Podcxl affects Jak3 basal activity of STAT3 in myeloid progenitors, and if Jak3 activity of STAT3 in the emergency granulopoiesis due to response to G-CSF administration.

Figure 3: Loss of Podcxl reduces frequency of GMPs during homeostasis and G-CSF-induced stress granulopoiesis. A) Representative gating panel showing expression of GMPs in bone marrow tissues from mice treated with PBS or G-CSF. B) Frequency of GMP population between Podcxl cko and wildtype mice treated with either PBS or G-CSF. Statistical significance was performed using the Student’s Two-Tailed T-test for Significance. *p<0.05 ***p<0.001.

Figure 4: Loss of Podcxl reduces activity of STAT3 during emergency granulopoiesis and homeostasis. These results coincided with reduced number of the GMP population in both Podcxl ko mice injected with G-CSF and those injected with PBS, suggesting that Podcxl deletion restricts GMPs’ differentiation.

Figure 5: Model of Podcxl’s role in G-CSF induced emergency Granulopoiesis. (Left panel) Podcxl is hypothesized to promote Jak3/STAT3 pathway. Jak3/STAT3 pathway consists of transcriptional signaling factors that control downstream-driven neutrophil production. Briefly, G-CSF phosphorylates transmembrane protein Jak2 which then phosphorylates STAT3. Newly activated STAT3 drives nucleus to activate transcription factors to promote reactive granulopoiesis, leading to production of neutrophils. Podcxl is expressed in hematopoietic systems (right panel) leads to decreased GMPs’ production in bone marrow, with concomitant drop in mature neutrophils (data not shown). Loss of Podcxl results in elevated accumulation of immature neutrophils in bone marrow, with the associated drop in mature neutrophils in the bone marrow and periphery. This results in the need for increased neutrophil levels in the absence of Podcxl during G-CSF induced response remains to be elucidated.

References


Acknowledgements

This research was supported by the SSRF program; thanks to Dr. Koz, Dr. Liow and Liz Burt. Thank you Dr. Sathyanarayana and Dr. Karaczyn for your guidance and support. MMCRI flow cytometry Core was used in this project.
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 cases 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=8,786)
- Final Cohort (N=165,674)

Baseline Characteristics of Cohort

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

Study Variables

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].

Odds Ratios

Without Covariates

With Covariates

Q1 vs Q2

Q1 vs Q2

Q1 vs Q2

Lower mortality

Higher mortality

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.
Focality and Co-infection of Powassan Virus (Lineage II) within a Population of Ixodes scapularis Ticks in Wells, ME

Keenan Ernste, Kyle Timmer, Rebecca Robich, Susan Elias, Charles Lubelzyk, Elisabeth Henderson, Danielle Cosenza, Margaret Welch, Robert Smith

Vector-Borne Disease Laboratory – Maine Medical Center Research Institute

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 (POVV) 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 (foci) consisting of just the right mixture of environmental factors for the virus to propagate. Exits primarily in a zoonotic cycle between Ixodes scapularis ticks (deer ticks) and small-medium mammals (e.g. the white-footed mouse, woodchucks, and skunks).

- 10% of POWV human infections result in death, and 50% result in long-term neurological sequelae.
- In the United States there has been a threefold increase in recent years.

Objectives

- Determine what percentage of DTV-infected ticks were also infected with Lyme, anaplasmosis, babesiosis, and/or tick-borne relapsing fever.
- Provide shade for ticks.
- DTV-infected ticks will exhibit nidality (focality).
- Co-infection will be observed in ticks infected with DTV.

Methods

Field Collection

- Ticks were 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 100 m plots.

Field Laboratory Testing

- 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

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

Results

Table 1: Field Site Characteristics and Collection Results

<table>
<thead>
<tr>
<th>Transect Number</th>
<th>Habitat Type</th>
<th>Number of DTV-Positive Ticks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Forest</td>
<td>23 (8.2)</td>
</tr>
<tr>
<td>2</td>
<td>Forest</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>3</td>
<td>Forest/Edge</td>
<td>113 (30.2)</td>
</tr>
<tr>
<td>4</td>
<td>Field</td>
<td>61 (16.0)</td>
</tr>
<tr>
<td>5</td>
<td>Field</td>
<td>10 (2.7)</td>
</tr>
<tr>
<td>6</td>
<td>Field/Edge</td>
<td>14 (3.5)</td>
</tr>
<tr>
<td>7</td>
<td>Field/Edge</td>
<td>21 (5.6)</td>
</tr>
<tr>
<td>8</td>
<td>Field/Edge</td>
<td>57 (18.0)</td>
</tr>
<tr>
<td>9</td>
<td>Field/Edge</td>
<td>11 (2.9)</td>
</tr>
<tr>
<td>10</td>
<td>Field/Edge</td>
<td>373 (10)</td>
</tr>
</tbody>
</table>

Results

Fig. 2. Left image is a map of human POWV/DTV infections in Maine and the right image is showing prevalence of POWV-infected deer ticks.

The 70% positivity rate for DTV and B. burgdorferi is suggested to be an indicator of elevated human risk of exposure to Ixodes scapularis in southern Maine and associated with berry ripening.

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. Positive ticks were also tested for co-infection with B. burgdorferi, A. phagocytophylum, B. microti, and B. miyamotoi.

- 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 0.6-5.6% range expected from previous reports. This 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 line II (DTV) and not lineage I (POVV) was found in I. scapularis ticks. 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).

References


Acknowledgements

The author thanks everyone in the Vector-Borne Disease Laboratory of the Maine Medical Center Research Institute for all of their guidance and support throughout this study.

https://knowledgeconnection.mainehealth.org/jmmc/vol1/iss1/16
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 commonly being researched in the biomedical field for its role in a variety of different health interests, including, but not limited to cancer, heart disease, inflammation, and a variety of other conditions [1]. CTHRC1 is a secreted hormone, and the proteolytic processing of this hormone, and where it is active and secreted, is essential information to use (CTHRC1) to further understanding of its role in these complex medical interests. The proteolytic processing of CTHRC1 is not clear, and we are specifically asking whether or not the hormone needs to be activated and if it does how it is being activated and secreted. CTHRC1 is expressed in neuroendocrine cells, which require regulated secretion to be activated and this is the task to further understand if CTHRC1 might be influenced by secreted and regulated secretion as well. As CTHRC1 has a terminal lysine, therefore on the CTHRC1 processing pathway, which has been previously described [2], leaving the terminal lysine, which is necessary for CTHRC1 to be secreted, allows us to characterize the proteolytic processing of CTHRC1 [3]. To characterize this mechanism, we used four cell lines. Each cell line was transfected with either control, hFL, hFLΔK, or VVD. We then probed these samples with various CTHRC1 antibodies, which included: observation of cell line that expressed endogenous CPE (AtT20) and the activation and secretion of CTHRC1 in these cells, including that of the cells transfected with the plasmid that has the myc tag did not secrete a higher amount of CTHRC1 in the conditioned medium. The control indicates non-specific bands that are similar to the bands that show the presence of CPE (exposed in the CL and CM, which could be explained by the presence of CPE, which is expressed in the cell lysate). These bands are much higher in the CL and CM, with the mouse CPE, which has a myc tag, shows a fully active sample. The bands that have an epitope travels further, which is caused by the CPE+ samples that have the PLZ bands. There is also another notable shift shown in the (B) sample, which is caused by the C THRC1 samples that have the trimer (the top band is significantly higher than that without CPE). This shift is significant in the mouse CTHRC1 samples, which has not been fully modified. There is also another shift that is not entirely clear, which is shown in the B) sample due to the presence of CTHRC1, which makes a shift in the CL.

Methods:

Transfection: The 293-T cells were transfected with various plasmids using Transfection Reagent. The cells were transfected when they were around 50-70% confluent. Transfection Reagent and pCMV-hFL were mixed and added to the wells, followed by addition of 25% of the conditioned medium. The conditioned medium was then added to the wells, and the cells were harvested in samples that were probed with various CTHRC1 antibodies. There are a series of shifts that are observed in these samples, which include: observation of cell line that expressed endogenous CPE (AtT20) and the activation and secretion of CTHRC1 in these cells, including that of the cells transfected with the plasmid that has the myc tag did not secrete a higher amount of CTHRC1 in the conditioned medium. The control indicates non-specific bands that are similar to the bands that show the presence of CPE (exposed in the CL and CM, which could be explained by the presence of CPE, which is expressed in the cell lysate). These bands are much higher in the CL and CM, with the mouse CPE, which has a myc tag, shows a fully active sample. The bands that have an epitope travels further, which is caused by the CPE+ samples that have the PLZ bands. There is also another notable shift shown in the (B) sample, which is caused by the C THRC1 samples that have the trimer (the top band is significantly higher than that without CPE). This shift is significant in the mouse CTHRC1 samples, which has not been fully modified. There is also another shift that is not entirely clear, which is shown in the B) sample due to the presence of CTHRC1, which makes a shift in the CL.

Results:

1. 293-T cells do not secrete CTHRC1 efficiently, even with the addition of CPE.
   2. In the cell lysate, the lower, dense band is the non-glucocorticoid version, whereas the upper band is the glucocorticoid version.
   3. In the AtT20 and CHO cells transfected with hFL and FDLK, there was the indication of a slight higher band in the conditioned medium of hFL and FDLK, 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 show nonspecific bands that are specific to that individual cell line.
   5. In the AtT20 cell transfection Western Blot there is a secretion outside of the cell especially with the difficulties in reading the Western Blot due to the nonspecific bands of a similar size, which was unexpected. Assuming that there were no difficulties with the transfection that indicates that this construct is only minimally able to be secreted.
   6. Posttransfection under mass spectrometry determined that CHO cells CTHRC1 has a terminal lysine, and so the result of CTHRC1 not having expressed endogenous CPE does not disproving the hypothesis.
   7. Holden in the CHO transfection, the samples transfected with 2O/myc, show no shift between the CM and CL. This may be assumed by the e-lterminal tag influencing the post-translational modifications that take place outside of the cell.
   8. Along with this observation, the O/myc Western Blot that indicates the expression of the CTHRC1, while the CM in 293-T cells indicate that the e-lysine is removed with the ability that it is shown. This cell line is not able to secrete CTHRC1 efficiently.
   9. In the 293 T cells when cotransfected with CPE+FLDLK, the top band is slightly higher than that of CPE+FLDK and appears to be a trimmer, which is unlikely after the alignment observed in Western Blot. This observation currently does not have a conclusion aside from the possibility that the terminal tag is missing, but will continue to be explored in future experiments.

Acknowledgements:

The work presented in this poster is possible without the support of the members of the Lindner Lab: Volkhard Lindner MD PhD, Yoon-Ji Lee, Jin PhD, Akira Matogawa, and Mayasah Al Hashimi, and many of the faculty of MMCRI with all of their support, expertise, and patience. The funding for this research is provided with grants thanks to Maine Medical Center Research Institute Summer Student Research Program and to the American Heart Association. Thank you so much for this opportunity!

References:

et al.: 2018 MMCRI Posters

1. 293- T cells do not secrete CTHRC1 efficiently, even with the addition of CPE.
2. In the cell lysate, the lower, dense band is the non-glucocorticoid version, whereas the upper band is the glucocorticoid version.
3. In the AtT20 and CHO cells transfected with hFL and FDLK, there was the indication of a slight higher band in the conditioned medium of hFL and FDLK, 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 show nonspecific bands that are specific to that individual cell line.
5. In the AtT20 cell transfection Western Blot there is a secretion outside of the cell especially with the difficulties in reading the Western Blot due to the nonspecific bands of a similar size, which was unexpected. Assuming that there were no difficulties with the transfection that indicates that this construct is only minimally able to be secreted.
6. Posttransfection under mass spectrometry determined that CHO cells CTHRC1 has a terminal lysine, and so the result of CTHRC1 not having expressed endogenous CPE does not disproving the hypothesis.
7. Holden in the CHO transfection, the samples transfected with 2O/myc, show no shift between the CM and CL. This may be assumed by the e-lterminal tag influencing the post-translational modifications that take place outside of the cell.
8. Along with this observation, the O/myc Western Blot that indicates the expression of the CTHRC1, while the CM in 293-T cells indicate that the e-lysine is removed with the ability that it is shown. This cell line is not able to secrete CTHRC1 efficiently.
9. In the 293 T cells when cotransfected with CPE+FLDLK, the top band is slightly higher than that of CPE+FLDK and appears to be a trimmer, which is unlikely after the alignment observed in Western Blot. This observation currently does not have a conclusion aside from the possibility that the terminal tag is missing, but will continue to be explored in future experiments.

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

Published by MaineHealth Knowledge Connection, 2019
Genetic Testing in Hospitalized Children with ASD: Prevalence and Influences
Margaret Merve1, Briana Taylor1,2, Christine Peura1,2, Matthew Siegel1,2
for the Autism and Developmental Disorders Inpatient Research Collaborative (ADIRC)

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.1,2
- Children with ASD seeking care in inpatient psychiatric units could benefit greatly from genetic testing due to the complex nature of their diagnoses and behaviors, 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 genetic testing were included in this study.
- Bivariate logistic regressions were conducted to examine potential factors within demographics, psychiatric unit, medical history, family history and behavior that could influence access, referral and reporting of genetic testing.

Results

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

<table>
<thead>
<tr>
<th>Demographic/Genetic Testing</th>
<th>No Genetic Testing (n=415)</th>
<th>Specialized Inpatient Unit (n=314)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years) (ASD)</td>
<td>13.32 (5.45)</td>
<td>12.4 (3.34)</td>
</tr>
<tr>
<td>Sex (Male) (N/%)</td>
<td>238 (57.5%)</td>
<td>150 (48.1%)</td>
</tr>
<tr>
<td>ADDS Module (N/%)</td>
<td>104 (5.5%)</td>
<td>125 (15.5%)</td>
</tr>
<tr>
<td>Parental Education (N/%)</td>
<td>235 (56.5%)</td>
<td>339 (75.1%)</td>
</tr>
<tr>
<td>Household Income</td>
<td>38 (11.4%)</td>
<td>36 (9.7%)</td>
</tr>
<tr>
<td>Adopted (N/%)</td>
<td>34 (11.4%)</td>
<td>96 (30.5%)</td>
</tr>
</tbody>
</table>

- Of the total child sample, 588 (80.6%) were male and the average age was 12.9 (SD = 3.3).
- 436 (54.3%) children were non- or minimally-verbal (determined byADOS Module administered).
- There were statistically significant differences in gender, ADDS module, and household income between the two genetic testing groups.
- Those with genetic testing were more likely to have a 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.

- Percentage of Parents Reporting Genetic Testing: 43.07% of parents reported having genetic testing for their child prior to their admission.
- Bivariate logistic regressions were conducted to examine the relationship between genetic testing in hospitalized children with ASD.

Conclusions

- Participants whose child was female were 71% more likely to report genetic testing (p < 0.004).
- Participants whose child was nonverbal (p = 0.005) or minimally verbal (p = 0.018) were 201% and 209% more likely to report genetic testing.
- Families with an income of less than $80,000 (p = 0.00) or $80,000 - $160,000 (p = 0.002) were 72.8% and 66.7% less likely to report genetic testing.

References


Acknowledgements

Funding for the AIC provided by a grant from The Simons Foundation and the Nancy Lurie Marks Family Foundation, Internship funded by Maine Medical Center Research Institute.
Physician-patient Communication About Genomic Tumor Testing: Perceptions Of Oncology Providers
Alexandra McCown, Caitlin Guthell, 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 the 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.

Background
• Genomic tumor testing (GTT) is a new technology and a cornerstone of the "precision medicine" movement in cancer care.
• GTT uses next-generation genome sequencing technology to identify somatic variants in tumor cells.
• By identifying somatic variants that predict responses to cancer therapies, GTT can help tailor therapy to individual patients, making them more effective.
• However, GTT also detects many variants of currently unproven value.
• When using GTT, physicians need to counsel patients about both its clinical and uncertainties about GTT.
• The Jackson Laboratory’s Maine Cancer Genomics Initiative (MCGI) is a 5-year statewide research project aimed at disseminating and implementing GTT in community oncology practices throughout the state of Maine.

Research Question
What are providers’ perceptions of the key goals and elements of physician-patient discussions about 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 tabulated.
• Analysis of open-ended items was conducted using qualitative methods.
  Software-assisted coding with MAXQDA™

Results
Participants
- Physicians: 17
- Oncology nurses: 14
- Genetic counselors: 5
- Pathologists: 5
- Other (e.g., practice administrators, genetic counselors, other physician specialists): 35
- Total: 76

Key Goals of Discussions
- Discuss nature of GTT
- Convey uncertainty about GTT
- Maintain patient hope
- Encourage patients to undergo genomic tumor testing
- Share your personal viewpoints on genomic tumor testing

Illustrative Quotations from Open-ended Responses
- "Every cancer is unique / This is a way to use precision medicine and offer a more personalized treatment based on cancer genetics 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"
- "Emerging technology: some meaning well understood, some not"
- "There may be no information that helps at this time but potential for future treatment"

Key Content Elements of Discussions
Nature of GTT (n=201)
Uncertainty about GTT (n=59)
Potential Outcomes of GTT (n=256)
Uncertainty about Therapeutic Options (n=26)
Uncertainty from Incomplete Evidence (n=10)

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.
  - Nature of GTT: Uncertainties about its value for treatment decisions, and patient expectations.
  - Key elements regarding uncertainty in GTT focus mainly on therapeutic options and incomplete evidence.

References

Acknowledgments
• Alexandra McCown was generously supported by the Korkel 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)

Illustrative Quotations from Open-ended Responses
- "Every cancer is unique / This is a way to use precision medicine and offer a more personalized treatment based on cancer genetics 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"
- "Emerging technology: some meaning well understood, some not"
- "There may be no information that helps at this time but potential for future treatment"

Illustrative Quotations from Open-ended Responses
- "Every cancer is unique / This is a way to use precision medicine and offer a more personalized treatment based on cancer genetics 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"
- "Emerging technology: some meaning well understood, some not"
- "There may be no information that helps at this time but potential for future treatment"

Illustrative Quotations from Open-ended Responses
- "Every cancer is unique / This is a way to use precision medicine and offer a more personalized treatment based on cancer genetics 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"
- "Emerging technology: some meaning well understood, some not"
- "There may be no information that helps at this time but potential for future treatment"
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 Robich1,2, 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 2,000g 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 micron filter.

Figure 2. The number of circulating microparticles in CA subjects at 6 hours is significantly higher compared to 24 hours and 72 hours after ROSC. Control (CABG, n=36) patients do not have a significant difference in the number of circulating microparticles compared to any of the CA subjects (n=44). Statistical significance was calculated using one-way ANOVA, p values from Dunn’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 (control, 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 Dunn’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.
**Abstract**

Research on the relationship of skeletal physiology and the nervous system has exploded in the last 25 years. Studies of β-adrenergic ligands and bone density have well established an inverse relationship between sympathetic nervous output and bone metabolism (Figure 1). Recently, the role of sensory nerves have also been considered in this story. The precise mechanisms of neuro-osteogenic crosstalk remain obscure. A better understanding of innervation with respect to bone remodeling will inform treatment of bone pathologies and disease. We use immunohistochemical staining to elucidate the phenotypes, distribution, and organization of nerve fibers in bone. Semiconductor nanocrystals (quantum dots) have more desirable optical qualities than organic dyes and fluorophores, and will be used to overcome the inherent difficulty in confocal imaging of bone.

**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 turn limits the ability to perform these techniques routinely. An efficient, reproducible technique for visualizing and quantifying the differential innervation along periosteum of femur. Tropomyosin (1) conjugated to secondary antibodies (Figure 3) was used to overcome the inherent difficulty in confocal imaging of bone.

**Methods and Materials**

- Fixed, frozen tissue sections of long bones and L5 vertebrae from transgenic mice expressing green fluorescent protein driven by sequence under trpm8 promoter. Both innervation along periosteum of femur and Brain3-cre mice were used to overcome the inherent difficulty in confocal imaging of bone.
- Conventional IHC performed to visualize neural markers and trpm8 with fluorescent markers.
- Quantum dots (Figure 4) conjugated to secondary antibodies (Figure 3). In lieu of organic fluorescent markers, 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.

**Results**

- Inconclusive staining for sympathetic innervation in growth plate of Tibia/Fibula (Figure 7). Inconclusive staining for sympathetic innervation along periosteum of femur (Figure 6).
- Multiplex imaging (Figure 8) of multiple neural markers and receptor (TH, CGRP, TRPM8) to show co-localization, gap due to communication mechanisms.
- Multiplex imaging (Figure 9) of neural density and density between long bones and vertebrae.
- Study changes in neural density from neuropathy in mouse BTBR Ob/Ob diabetes model.

**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 optimize concentration, antibody type/dilution/incubation times to optimize staining. Rigorous negative controls and positive controls (tissue donors from normal donors) 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.

**Contact**

Nicholas Banks

Nicholas.banks@maine.edu

**References**

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 and vascular health**

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.

**Gene candidate selection**

Proteomic data highlighted differential expression of proteins in perivascular adipose tissue (PVAT) of mice fed a high fat diet (HFD) versus those fed a control diet (CD). Twelve candidate genes with significantly different protein levels were identified as potential protein markers unique to perivascular adipose tissue.

Candidates were divided into two categories: those proteins upregulated in gonadal white adipose tissue (gWAT), brown adipose tissue (BAT), and PVAT in mice fed a high fat diet; and proteins upregulated in BAT and PVAT and downregulated in gWAT of mice fed a high fat diet. Transcriptome verification via qRT-PCR of the first category was performed.

**Methodology**

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

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>gWAT CD</th>
<th>gWAT HFD</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>
</tr>
</thead>
<tbody>
<tr>
<td>27A2</td>
<td>Very long-chain acyl-CoA synthetase</td>
<td>1,041</td>
<td>6,294</td>
<td>19,618</td>
<td>33,436</td>
<td>2,436</td>
<td>16,485</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMCS2</td>
<td>Hydroxymethylglutaryl-CoA synthase</td>
<td>6,773</td>
<td>50,776</td>
<td>2,399</td>
<td>6,749</td>
<td>2,115</td>
<td>11,594</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F11N2</td>
<td>Perilipin-2</td>
<td>16,034</td>
<td>219,927</td>
<td>19,137</td>
<td>31,808</td>
<td>14,437</td>
<td>41,056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NXN1A</td>
<td>Annexin A1</td>
<td>1,311,053</td>
<td>2,012,226</td>
<td>84,678</td>
<td>138,520</td>
<td>49,419</td>
<td>132,267</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS1D2</td>
<td>Hydroxysteroid dehydrogenase-like protein 2</td>
<td>142,617</td>
<td>156,995</td>
<td>317,538</td>
<td>475,215</td>
<td>136,610</td>
<td>309,144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG1L2</td>
<td>Transgelin-2</td>
<td>773,866</td>
<td>1,083,330</td>
<td>198,420</td>
<td>293,259</td>
<td>123,726</td>
<td>196,127</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Candidate proteins displaying differential abundance from protein mass spectrometry of adipose tissues.**

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

**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, with 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 Karoš (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 (REDCapTM).
- 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 = 0.017). 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.
- Additionally, 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 decapsulation 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.
3T3-L1 model of adipogenesis and effects of methionine restriction

Sharon Jordan, Emily Cooper, Lucy Liaw PhD
Maine Medical Center Research Institute, Scarborough, Maine.
Sharonajordan@smccme.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. Perivascular 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 aspirated off and allowed to dry. Lipid rich areas were stained red. The ORO solution was removed, and cells rinsed with diH2O twice followed by 3x10 minute diH2O washes. 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.

Results

3T3-L1 model of adipocyte differentiation

Figure 1. Adipogenic differentiation of 3T3-L1 cells
a) 3T3-L1 cells before differentiation. b) 3T3-L1 cells after successful differentiation and lipid stained with oil red O.

Effects of methionine modification on adipocyte cell number and differentiation

a) Absorbance of crystal violet dye was measured to estimate the amount of cells that survived under each methionine condition. b) Oil Red O was used to stain the lipid. Amount of Accumulated Oil Rd O was normalized to amount of CV in corresponding cells.

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 tool 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...
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 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

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.

Adipogenic

The Liaw lab’s Joshua Boucher, PhD, has been able to explant progenitor cells from these human PVAT samples and successfully induce them to differentiate 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 PLIN1 or perilipin 1. However, a major question still remains: How committed are these progenitor cells yielded by explantation of human thoracic PVAT to an adipogenic lineage? This project seeks to begin to

Osteogenic

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

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 100 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.
- The only curative treatment at this time is resection, yet only 10-20% of patients are considered clinically resectable at the time of presentation.
- 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.
- Studies have shown that receiving chemotherapy at some point is advantageous compared to just surgery, but the sequence is debated.

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, T1N2M0, T2N0M0, T2N1M0, T2N2M0
  - Patients undergoing palliative care were excluded
  - Patients who refused surgery were excluded
- Descriptive statistics were used analyze the data

Results

- Though chemotherapy before surgery has a slightly higher 5 year survival rate when compared to the surgery first group, the dropout rate is such that 72.1% of patients never make it to surgery, thus never giving them a chance for a cure.
- Using the decision tree, there is a 13.1%, 5 year survival benefit to receiving surgery first.
- Using Cox Regression analysis, patients were more likely to receive surgery if they had private insurance, were at an academic center, and lived in a metropolitan area.
- Patients were less likely to receive surgery first, if they were older than 65, male, had multiple comorbidities, had a poor tumor grade, had a tumor of the pancreatic head or neck.

References


Variable | HR (95% CI) | P value
--- | --- | ---
Age, 65-79 | 1.15 (1.09-1.21) | <0.0001
Age, 85-99 | 1.62 (1.56-1.77) | <0.0001
Male | 1.05 (1.01-1.08) | 0.004
White | 1.02 (1.01-1.05) | 0.039
Private insurance | 0.88 (0.83-0.93) | <0.0001
Academic center | 0.79 (0.77-0.82) | <0.0001
Metropolitan area | 0.92 (0.80-1.03) | 0.007
Comorbidities | 1.15 (1.11-1.18) | <0.0001
Poor tumor grade | 1.26 (1.21-1.31) | <0.0001
Head/Neck Tumor | 1.09 (1.06-1.14) | <0.0001
Start with Surgery | 0.48 (0.46-0.50) | <0.0001

Decision Tree

- Not resectable for either
- Receptor 1
  - Chemotherapy First
  - Survival 55%
- Receptor 2
  - Surgery First
  - Survival 22%

- chemotherapy first vs upfront with adjuvant chemotherapy

Prospective, randomized controlled trials comparing neoadjuvant therapy against an upfront surgical approach are needed to better answer this question.

Next Steps

- randomized controlled trials comparing neoadjuvant therapy against 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. Helderaman, Liv Palma¹, and Clifford J. Rosen¹

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

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.
- Cholesteryl 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 tissues², as well as defective brown adipose tissue, leading to hypothermia at room temperature.³

Recently, our lab has demonstrated LALKO mice also have decreased bone volume/total volume (BV/TV) in the distal femur metaphysis.

We hypothesized that LAL supports osteoblast differentiation.

METHODS

Bone Marrow Stromal (BMSC) Cell Culture

- C57BL/6 mice were sacrificed and their femurs, tibias, 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 10⁶ cells per well in three 12-well plates.

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 lalitstat group of bone marrow stromal cells

REFERENCES

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

Methods

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.3 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 thresholds1,2 in order to predict a person's neurological outcome. We did this by looking at three time intervals: 6 hours after return of spontaneous circulation (ROSC), 24 hours after ROSC, and ever. Our hypothesis was that constriction velocity would be a more accurate predictor of neurological outcome than NPi and size.

Introduction


Acknowledgements

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

References

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. 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.

HYPOTHESIS
Electrical 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.

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.

RESULTS
The average visit duration for those utilizing chemical cardioversion was 331.8 minutes, whereas the average duration for those only undergoing electrical cardioversion was 206.67 minutes.

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.

Table 1: Percentage of patients experiencing complications after electrical cardioversion procedures within the emergency department (n = 680)

<table>
<thead>
<tr>
<th>Complication</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR &lt; 50 bpm</td>
<td>3.4 %</td>
</tr>
<tr>
<td>Ventricular Tachycardia</td>
<td>1.8 %</td>
</tr>
<tr>
<td>Systolic BP &lt; 90 mmHg</td>
<td>9.2 %</td>
</tr>
<tr>
<td>O2 saturation &lt; 90%</td>
<td>4.0 %</td>
</tr>
<tr>
<td>Intubation/Bagged</td>
<td>0.7 %</td>
</tr>
</tbody>
</table>

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

REFERENCES

ACKNOWLEDGEMENTS
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, correlates 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 microRNAs that may harbor anti-diabetic properties. We found that miRNA-32, a microRNA 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 microRNAs 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 microRNA content.

Methods

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

Results

Isolation of exosomes from brown adipocytes
Exosomes were isolated from cultured adipocytes and their exosomes were analyzed using flow cytometry to determine their presence in exosome surface markers CD9 and CD61.

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 adipocytes exosomes showed a gel band in the approximate range expected for miRNAs (20-200 base pairs).

Amplification of microRNAs from exosomes
microRNAs were extracted from exosomes isolated from brown adipocytes using the Qiagen miRNeasy Micro Kit. microRNAs were converted to cDNA using gene specific reverse transcription primers for 3 exosomal microRNA markers previously shown to be expressed by brown adipocytes, including miRNA-92a, miRNA-99b and U6 snRNA. U6 snRNA is a non-coding small nuclear RNA commonly used as internal control to normalize microRNA 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 expression was previously shown to be necessary for activation of brown adipocytes (Cell Rep. 2017, 19(6):1229-1246). We found that this microRNA 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 against sequencing microRNAs from exosomes isolated from cell cultures of brown adipocytes in our search for molecules that may regulate type 2 diabetes.

Acknowledgements
This work was supported by NIH COBRE award P20GM121301 (A. Brown, L. Liaw, and C.J. Rosen). The project utilized services of the Molecular Phenotyping and Progenitor Cell Analysis Core Facility funded by the NIH COBRE award P20GM106391 (R. Friesel, PI).
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 oncogenetic 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).
• Etoricoxib (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 etoricoxib has been proven to reduce cancer cell viability and proliferation.
• Recently etoricoxib 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 etoricoxib 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

2. In vitro Combination Treatment of Etoricoxib and Bortezomib

A OPM2: Etoricoxib + Bortezomib

B MM1R: Etoricoxib + Bortezomib

Cell viability of (A) OPM2 and (B) MM1R cells treated with etoricoxib (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.

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

MM15 cells were directly co-cultured with mouse mesenchymal stem cells (mMSCs) for 24 hours before etoricoxib (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, and the number of viable cells was measured 72 hours after treatment.

METHODS

Cell Culture: MM15 (gfp+/luc+), MM1R (gfp+/luc+), and OPM2 (mcherry/luc+) were seeded at various densities into clear and white 96 well plates.

Drug administration: Etoricoxib (CPT1 inhibitor), Bortezomib (proteasome inhibitor), and Dexamethasone (corticosteroid that reduces inflammation) were added at various doses 24 hours after the cells were seeded. Bortezomib and dexamethasone are common pharmacological anti-myeloma treatments.

Cell Number: Bioluminescent imaging (BLI) was used to quantify cell viability. Luciferin [10 µL] was added to each well and incubated for 15 minutes before reading with Glomax® Microplate Reader. BLI was read after 24, 48, 72, and 96 hours after the drugs were administered to the cells.