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 https://doi.org/10.46804/2641-2225.1017

The views and thoughts expressed in this manuscript belong solely to the author[s] and do not reflect the opinions of the Journal of Maine Medical Center or MaineHealth.

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.
The Role of Lipid Metabolism in Multiple Myeloma

DeSchiffart, Abigail; Masarwi, Majdi; Reagan, Michaela R.;

1Maine Medical Center Research Institute, 2Tufts University School of Medicine, 3University of Maine Graduate School of Biomedical Science and Engineering

INTRODUCTION

Multiple Myeloma (MM) is the second most common type of hematological cancer, formed from a series of oncogenic mutations to the plasma cells of the bone marrow (1). Initially patients respond well to chemotherapeutic treatment, but almost all eventually develop resistance to these treatments and experience relapse.

Myeloma thrives in the unique and complex bone marrow microenvironment. Also, within the bone marrow are bone marrow adipocytes (BMA) that form bone marrow adipose tissue and account for 50-70% of the total bone marrow volume. It is believed that BMA promotes 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 mitochondria. (3) 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 complex one of the electron transport chain at high dosages (4).

In addition to a potential energy source, BMA has been shown to increase MM’s resistance to chemotherapeutic treatments (5). • We are examining the effect of etomoxir on different MM cell lines and if it increases MM sensitivity to other 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.

Cell Culture: MM1S (gfp+/luc+), MM1R (gfp+/luc+), and OPM2 (mcherry/luc+) were seeded at various densities into 96 well plates.

Drug administration: Etomoxir (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 commonly used chemotherapeutic treatments.

RESULTS

1. In vitro Combination Treatment of Etomoxir and Bortezomib

MM1S cells were directly co-cultured with mouse mesenchymal stem cells (mMSCs) for 24 hours before etomoxir (5µM) and dexamethasone (0.5µM) were administered. The MM1S cells were seeded at a cell density of 7500 cells/well. The MM1S cells were seeded at a cell density of 5000 cells/well. Statistical analysis was done using one way ANOVA test. *p<0.05; **p< 0.0001.

2. In vitro Combination Treatment of Etomoxir and Bortezomib

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

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

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

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

CONCLUSIONS

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

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

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

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

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

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

REFERENCES

1. Keh, R. A., & Rajkumar, S. V. (2008). Multiple myeloma. Blood, 111(10), 2602-2627.

2. Fassler, P. K. et al. (2015). Macronutrient and Bone—New Perspectives. J. Clin. Endocrinol. Metab. 99: 325–335.

3. Garcia, M. I., Shiha, G. O., Parker, J. S., Fan, C., & Parviz, M. (2014). An integrated genetics approach identifies drivers of proliferation in luminal-subtype human breast cancer. Nature Genetics, 46(11), 1051-1059. DOI: 10.1038/ng.3207.

4. Ip, H., Liu, T., Wang, J., Yan, X., & Ram, S. (2018). Identifying off-target effects of etomoxir reveals that carnosine palmitoyltransferase I is essential for cancer cell proliferation independent of β-oxidation. PLOS Biology 16(11): e2003782.

5. Falahati, C., Fairhall, H., Farrell, M., & Reagan, M. (2017). New Bone Cell Type Identified As Driver of Drug Resistance in Multiple Myeloma: The Bone Marrow Adipocyte. Blood 130.

6. Melone, M. A. S., Valenti, A., Maragno, S., Galderisi, U., Giordano, A., & Peluso, G. (2018). The carnitine system and cancer metabolic plasticity. Cell Death & Disease, 9(2), 228.

Conflicts of Interest: There are no conflicts of interest in this work to disclose.

https://knowledgeconnection.mainehealth.org/jmmc/vol1/iss1/16
DOI: 10.46804/2641-2225.1017
Loss of miR-199b promotes expansion of myeloid-committed progenitors differentiated from bone marrow and spleen HSCs

Aidan McGrory¹, Aldona Karaczyn¹, Edward Jachimowicz¹, Pradeep Sathyanarayana¹
¹Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, Maine

Abstract

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

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

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

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

Experimental Approach

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

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

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

Discussion and Conclusions

In conclusion, our results indicate that attenuation of miR-199b activity promotes myeloid progenitor proliferation. We found that miR-199b deletion favors myeloid lineage commitment and expansion of myeloid progenitors when HSPCs were isolated either from BM or spleen, suggesting that these HSPCs features result from intrinsic changes rather than niche alterations. Reduced colony forming abilities of splenic 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. Holden 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 Jane Friedman for providing me with such a positive summer experience.

et al.: 2018 MMCRI Posters

Published by MaineHealth Knowledge Connection, 2019
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 afflicted patients. A major challenge in developing patient specific models is the complexity of the tumor microenvironment (TME). One important component of the TME is the extracellular matrix (ECM), which has not been well characterized in ccRCC. Here we sought to understand the composition and structure of ECM in individual patients. Tumor and matching normal ECM and cells were isolated from patient biopsies and characterized through LC-MS mass spectrometry and immunofluorescence. Independent data acquisition and analysis using SWATH identified drastic changes in collagen content between tumor and normal cells. It was further determined that a denatured collagen environment increased cell adhesion and Akt signaling using an in vitro cell adhesion assay with the 786-O ccRCC cell line. XL313, a truncated RGDFEG collagen epitope, was shown to be secreted by 786-O cells. Immunohistochemical staining of patient tumors showed apparent 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.

### Methods

#### Immunohistochemistry:
- **Differential adhesion:** 786-Os were seeded onto normal and denatured collagen IV. Cell adhesion was measured using a two well format. To determine the role of denatured collagen IV on cell adhesion, 786-Os seeded onto normal and denatured collagen IV were isolated from patient biopsies and characterized through LC-MS mass spectrometry and immunofluorescence. Independent data acquisition and analysis using SWATH identified drastic changes in collagen content between tumor and normal cells. It was further determined that a denatured collagen environment increased cell adhesion and Akt signaling using an in vitro cell adhesion assay with the 786-O ccRCC cell line. XL313, a truncated RGDFEG collagen epitope, was shown to be secreted by 786-O cells. Immunohistochemical staining of patient tumors showed apparent 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.

### Results

#### Conclusions

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

#### 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 other localization of collagen in ccRCC cell lines 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 on tumor growth in vivo**

### Acknowledgements

This project was funded by the National Science of Health. Thank you in advance for being a part of the following contributors to this project. Thank you to Dr. Sims-Lucas at the University of Pittsburgh for providing ccRCC tumor and normal tissue samples. We would like to thank the Maine Medical Center Research Institute for providing financial support.

**References:**

1. Hsieh, J. J. et al. Renal cell carcinoma. Nat. Rev. Dis. Primers 3, 17009 (2017).
2. Nabi, S., Kessler, E. R., Bernard, B., Flaig, T. W ., & Lam, E. T. (2018). Renal cell carcinoma: a review of biology and pathophysiology. Pathway. The Journal of Biological Chemistry, 291(6), 2731–2750. http://doi.org/10.1074/jbc.M115.669614
3. H. J. and O. J. B. (2016). Identification of a New Potentially Upregulated Epitope in Human Renal Cancer. PLoS ONE 11(4): e0152780. doi:10.1371/journal.pone.0152780
4. Stolnick, C. J. et al. Renal Cell Carcinoma. Curr. Rev. urology. 10(3): 200-209.
5. Deck, A. et al. Clear Cell Renal Cell Carcinoma: Characterization of the Tumor Extracellular Matrix. Anna Deck, Kyle Bond, Peter Brooks, Leif Oxburgh Maine Medical Center Research Institute
Incidence and Characteristics of Opioid-Related Cardiac Arrests at Maine Medical Center

Bailey West1,2, Teresa May3, John Dziodzio1, Tyler Nussinow1, Barbara McCrum3, Christine Lord4, Ashley Eldridge3, Deanna Williams1, Lee Lucas1, Philip Stone3, Richard R. Riker3, David B. Seder3

1Department of Critical Care Services, Maine Medical Center, Portland, ME; 2Honors College, The University of Maine, Orono, ME; 3Maine Medical Center Research Institute, Scarborough, ME; 4Center for Outcomes Research, Portland, ME.

Abstract

- The pathophysiology of ORCA differs from other cardiac arrest populations.
- A retrospective chart review was performed on medical records of MMC cardiac arrest patients and in prospectively collected registry data. ORCAs were identified using the following criteria: (1) the arrest was opioid-related; (2) the physician team suspected the arrest was opioid-related; or (3) an opioid-toxicology screen was ordered. Statistical analyses were used to compare characteristics and treatment outcomes between ORCA and non-ORCA patients.

Methods

- A retrospective chart review was performed on patients admitted to MMC for cardiac arrest from January 2013 to January 2017.
- Each arrest was classified as ORCA or non-ORCA using the criteria described in [1].
- The incidence of ORCAs at MMC has increased by 73% from 2013 to 2017.
- ORCA patients were younger with fewer comorbidities compared to non-ORCA patients.
- Additional cardiac interventions were more common in ORCA patients.
- There was no difference in the rate of in-hospital mortality and good functional outcome between ORCA and non-ORCA patients.

References

1. Rabbat E., et al. (2015). Recreational drug-related cardiac arrests at Maine Medical Center. Resuscitation, 91(Suppl A), S13-S20.
2. Seder DB., et al. (2018). The incidence of ORCAs at MMC has increased by 73% from 2013 to 2017.

Next Steps

- Characterize ORCAs geographically throughout Maine.

Acknowledgements

This research was supported by the Data and Video Repair Warrant (DVR) for Research Education and the Maine Medical Center Research Institute (MMCR).
Spry1 deficiency in mice shows fat depot specific alterations in adipose tissue responses to a high-fat Western Diet

Bridget Mellon1, Shivangi Pande2, 3, Xuehui Yang3 and Robert Friesel2, 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 "crown-like structures" around adipocytes that are indicators of fibrosis. We observed decreased coronal structures in the epididymal adipose tissue of Spry1 -/- mice fed a WD compared to Spry1+/+ mice. Our results also demonstrated that Spry1 -/- mice have a higher incidence of KLF4 and F4/80 positive cell staining compared with Spry1+/+ mice, indicating that the difference in fibrosis may be due to differential expression of the above markers. Thus, loss of Spry1 may be protective against Western Diet induced adipose tissue dysfunction.

INTRODUCTION

Atherosclerosis, an inflammatory disease characterized by arterial wall thickening and plaque accumulation, is a major cause of death worldwide. It presents itself as an inflammatory response initiated by activation of the endothelial layer surrounding the vessel walls and it proceeds to a proliferative and migratory, synthetic state.

Our lab has established that Spry1, a regulator of receptor tyrosine kinase (RTK) signaling, plays a critical role in the maintenance of the contractile VSMC phenotype of the smooth muscle cells. From a dormant, contractile state, to a proliferative and migratory, synthetic state.

RESULTS

Figure 1: X-gal staining of Spry1-/- mice showing expression of Spry1 in the adipocyte cells in eWAT and iWAT tissue.

Figure 2: H&E staining of eWAT showing less "crown-like" structures surrounding adipocytes of Spry1 -/- mice compared to Spry1+/+ mice.

Figure 3: Expression of KLF4 in eWAT and iWAT of HFD fed Spry1 -/- and Spry1+/+ mice

Figure 4: Expression of F480 in eWAT and iWAT for HFD fed Spry1 -/- and Spry1+/+ mice

CONCLUSIONS

- 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 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 hypercholesterolic 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.

REFERENCES

Yang, X., Gong, Y., He, G., et al., (2017). Loss of Spry1 attenuates vascular smooth muscle cell cycle regulatory circuits. Journal of Cellular Biochemistry, 119(4), 3267-3279. doi:10.1002/jcb.26486

Wayne Orr, A., Huang, N.E., Blackman, B.R., & Wamhoff, B.R. (2009). Complex Regulation and Function of the Inflammatory Smooth Muscle Cell Phenotype in Atherosclerosis. Journal of Vascular Research, 47(2), 168-185. doi:10.1159/000218093

Shankman, L. S., Gomez, D., Cherenkov, O. A., et al., (2016). Erratum: Corrigendum: KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. Nature Medicine, 22(2), 211-211. doi:10.1038/nm0216-211a

Acknowledgments

We would like to thank the MMCRI Animal Facility and the MMCRI Histology Core for their services (NIH grants P30 GM106391 (R. Friesel, PI), P20 GM113231, L. Lison, PI).
Crafting a resource guide for curriculum building in EM POCUS Continuing Medical Education

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

Abstract + Background

Ultrasonography (ultrasound) is a technology that uses high frequency sound waves to generate moving images of tissue. Point of care ultrasound (POCUS) is ultrasound brought to the patient and performed in real time. POCUS began in the 1990s as a tool used to improve diagnosis, procedures, and screening in multiple specialties. POCUS has become particularly important in emergency departments. This project explored the scope of POCUS training in emergency medicine (EM), compared ways that competency in POCUS is assessed, and used literature review to create an 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?
   - Determine “core” ED applications of POCUS
   - Review assessment tools available + their validity
2. Integrated feedback mechanisms: How will you determine what they learned?
   - Create a way to assess needs of physician population
3. Detailed instructional methods: How will you teach them this material?

Methods

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

Results

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

![Image](https://www.acep.org/sonoguide/)

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

Conclusions

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

Acknowledgments

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

References

- Henwood et al., Annals of Emergency Medicine, 2014
- Tolsgaard et al., PLoS One, 2013
- Ramsey et al.: 2018 MMCRI Posters

Published by MaineHealth Knowledge Connection, 2019
### 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.

### Methods

The study team is testing adolescents ages 12-21 who have recently experienced an episode of Grade 4HG. adolescents who have type 1 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.

### Introduction

#### Type 1 Diabetes

**Healthy**

- Normal glucose levels
- No symptoms during hypoglycemia

**Diabetic**

- Severe glucose tolerance
- Symptoms include fatigue, irritability, hunger

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

### Time Course of Cognitive Change after Severe Hypoglycemia

**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 impede 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)

Conceptual Models

- Precontemplation
- Contemplation
- Preparation
- Action

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

Literature Review

- Using TTM, patients educated according to their specific readiness stage are two times more likely to engage in a recommended health behavior in the next 6 months than those with generalized recommendations.
- Meta-analysis of 6 studies found that 40% of patients on dialysis lack health literacy in their ability to take care of themselves and change certain behaviors to support health.
- An online program (educating patients on sun protection after a kidney transplant) had direct ties to Skelton’s Recommendations (see Methods section). Patients with initial low health literacy made significantly greater gains in knowledge than patients with adequate health literacy after completing the program.

Prototype: A 5 Part Online Power Point with Audio

- Alternative Pathway for Education

Conclusions

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

References + Acknowledgements

1. Elwyn, Glyn et al. A Three-Talk Model for Shared Decision Making: Building Constructive Partnerships. 2017.
2. Swanson, Aaron. Trans Theoretical Model: Coaching and Cuing. 2015.
3. Skelton, SL et al. Applying best practices to designing patient education for peri-transplant care. 2015.
4. Lambert, Kelly et al. A Cross-Sectional Comparison of Health Literacy Deficits Among Patients With Chronic Kidney Disease, Journal of Health Communication. 2015.
5. Nielsen, MJ et al. Enhancing Access to Health-Literacy Materials of Chronic Care Management: A Randomized Controlled Trial. 2015.
6. Waterman, AD et al. Educating Prospective Kidney Transplant Recipients for Increasing Living Donation Rates. 2016.
7. Robinson JK et al. Response Across the Health-Literacy Spectrum of Deficits Among Patients With Chronic Kidney Disease, Journal of Health Communication. 2015.
8. Waterman, AD et al.: 2018 MMCRI Posters

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

A Review of C2 Nerve Root Transection Techniques

| Study & Year | Cauterization | Sectioning Location | ON (95% CI) |
|--------------|---------------|---------------------|-------------|
| Aryan (2008) | bipolar electrocautery (EC) with metzenbaum scissors | proximal to the DRG | 1 of 102 (p=0.010) |
| Squires and Molinari (2010) | monopolar EC | mid-portion of C2 articulation | 0 of 14 (p=0.000) |
| Kang et al (2012) | bipolar EC with Malis scissors | mid-portion of C2 articulation | 0 of 19 (p=0.000) |
| Yeom et al (2013) | monopolar EC; bipolar EC & knife or metzenbaum scissors | proximal to the DRG (or through the DRG) | 6 of 24 (p=0.250) |

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, transusions, or CSF leakage.

Results

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

| Early f/u Office Visits | # of Patients | # Nerves | Time Range | Occipital Pain | Narcotics for head pain? | Type of Narcotic |
|------------------------|---------------|----------|------------|----------------|--------------------------|----------------|
| 35                     | 66            | 1-7 months (mean=2.7) | 0 | 0 | n/a |

Delayed Phone Survey

| Phone Survey | # of Patients | # Nerves | Time Range | Occipital Pain | Narcotics for head pain? | Type of Narcotic |
|--------------|---------------|----------|------------|----------------|--------------------------|----------------|
| 17           | 31            | 0.5-4 years (mean=2.2) | 4 of 17 | 1 of 17 | liquid morphine |

Table 3. Results from delayed follow up patients

| Severe Neck Pain (≥ 3) | Severe Headaches (≥ 3) | Occipital Pain | QOL ≥ 6 |
|------------------------|------------------------|----------------|---------|
| 3 of 16                | 1 of 16                | 4 of 17        | 14 of 17 |

By M Walid et. al.

Figure 3. Demonstrates the portion of the head effected by the C2 nerve.

“Variation of Type III Odontoid Fracture As Isolated Jaw Pain” By M Walid 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
• 1 of the 4 patients was taking narcotics for head pain
• 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

Aryan, H. E., et al. (2008). “Stabilization of the atlantoaxial complex via C-1 lateral mass and C-2 pedicle screw fixation in a multicenter clinical experience in 102 patients: modification of the Harms and Goel techniques.” *Neurosurgery* 63(3): 222-229.

Kang, M. M., et al. (2012). “C2 nerve root sectioning in posterior C1-2 instrumented fusions.” *World Neurosurg* 77(1-2): 170-177.

Yeom, J. S., et al. (2013). “Postoperative occipital neuralgia with and without C2 nerve root transection during atlantoaxial screw fixation: a post-hoc comparative outcome study of prospectively collected data.” *Spine J* 13(7): 786-795.

Squires, J. and R. W. Malott (2010). “C2 ganglion mass excision with transoral sacrifice of the C2 ganglion: functional outcomes and morbidity in elderly patients.” *Spine J* 10(10): 1313-1318.
Characterization of a Novel Mouse Model with Adipocyte-specific Transgenic Expression of Mesoderm Specific Transcript

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

Abstract

Mesoderm Specific Transcript (Mest) is expressed variably in genotypically obese mice and highly induced in adipocytes when mice are fed a high-fat diet. The correlation of elevated Mest expression to increased body weight and fat mass gain raises Mest as a putative epigenetic regulator of diet-induced obesity. In order to determine if Mest expression in adipocytes can be regulated, a CAG promoter where the expression of Mest can be activated by tissue-specific expression of Cre recombinase. Transgenic mice were placed on a chow diet and the organs and fat pads were harvested. RNA was isolated from these tissues and analyzed by qRT-PCR in order to confirm that the transgenic model was tissue-specific. qRT-PCR analysis demonstrated that Mest expression was variably expressed in adipocytes. The induction of Mest in adipocytes of a transgenic mouse model was highly variable in response to a high-fat diet. When Mest was knocked out in adipocytes of this transgenic model, the correlation of elevated Mest expression to increased body weight and fat mass was abolished. This study indicates that Mest plays a role in the biological mechanism by which diet affects adipose tissue weight.

Introduction

Epigenetics & Obesity

Epigenetics is defined as the study of how changes in an organism's physical or environmental environment affect phenotypic traits without changing the underlying DNA sequence. In mice, obesity is a heritable trait in genetically obese strains such as db/db and ob/ob. There is evidence that obesity is a multifactorial disease in which both genetic and environmental factors contribute to the development of diet-induced obesity. Using an inbred, genetically homogeneous model of diet-induced obesity, we aimed to test the hypothesis that epigenetic regulators for obesity exist.

Epigenetic expression of Mest is co-regulated with several pathways. Using a transgenic model, we were able to confirm that Mest expression is induced in adipocytes when mice are fed an obesogenic diet. The correlation of elevated Mest expression to increased body weight and fat mass was abolished when Mest expression was knocked out in adipocytes of this transgenic model. This study indicates that Mest plays a role in the biological mechanism by which diet affects adipose tissue weight.

Methods

Transfection of 3T3-L1 cells with CAG-GFP-Mest and CAG-GFP-Mest-Myc (A,B) and transfection of cells with CAG-cre recombinase (C,D) resulted in reduced GFP signal for CAG-GFP vectors. Cells were quantified by qRT-PCR.

Summary/Conclusions

To better understand the function and regulation of Mest in adipose tissue expansion, we generated a conditional CAG-FGF-Mest transgenic mouse model that overexpresses Mest only in adipocytes. We determined that mice with transgenic Mest expression showed increased levels of Sfrp5 and Slc5a7 in gonadal fat and its brown fat, and lower levels of Ucp1 that was most evident in inguinal fat.

Acknowledgements

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

Supported by pilot funding from P20GM123131 (COBRE, Line)
Podocalyxin Promotes Activation of STAT3 Signaling Pathway in Emergency Granulopoiesis

Jahanara Freedman,1,2, Aldona Karaczyń and Pradeep Sathyanarayana1

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

Abstract

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

Rationales: 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 Podcalyxin (Podxl) had significantly elevated peripheral blood neutrophils following G-CSF treatment. Therefore, the long-term goal of this project is to understand how Podxl regulates granulopoiesis and functional maturation of neutrophils in bone marrow. In this study, we investigated whether loss of Podxl affects Jak3 activity of STAT3 in myeloid progenitors, and if any activity of STAT3 in the emergency granulopoiesis due to response to G-CSF administration.

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

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

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

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

Acknowledgements

This research was supported by the SSRP program; thanks to Dr. Kozak, Dr. Liow and Liz Bergst. Thank you Dr. Sathyanarayana and Dr. Karaczyń for your guidance and support. WMEC flow cytometry core was used in this project.

References

Cohen LG, Lai R, Gierasch L, et al. STAT3 activation in granulopoiesis is required for G-CSF-dependent differentiation. Immunity 2002;17(1):63-72

Figure 1: Flowchart diagram portraying the signal transduction approaches and gating strategy was used as described in Figure 2. Anti-STAT3, Phospho-STAT3 PE and isotype control antibodies were also used. Statistical significance was performed using the Student's Two Tailed T-Test for Significance. *p<0.05 ***p<0.001

Figure 2: G-CSF induces GMP proliferation and STAT3 activation in vitro. Lineage negative c-kit positive, CD15-negative compartment (G-CSF) containing myeloid progenitors from wildtype mice mixed with either G-CSF or PBS in bone marrow lineage negative population, CD34 and CD16/32 antibodies were used. GMP compartments were further analyzed to show expression of phosphorylated STAT3 in unpertubated STAT3 in wildtype mice injected with either PBS or G-CSF. In bone marrow, lineage negative population, CD34 and CD16/32 antibodies were used. Plots were collected using FlowJo. Statistical significance was performed using the Student's Two-Tailed T-Test for Significance. *p<0.05 ***p<0.001

Figure 3: Loss of Podxl reduces frequency of GMPs during homeostasis and G-CSF-induced stress granulopoiesis. GMPs, neutrophils, and myeloid progenitors were analyzed in bone marrow of WT and Podxl cKO mice. Mice were injected with 125 μL G-CSF twice in two days to induce emergency response in hematopoietic system. Initially, red of myeloid progenitors (GMPs) were tested in wildtype and wildtype G-CSF treated animals. Tested groups included wildtype(WT), wildtype with G-CSF(WT-G-CSF), Podxl xKO(WT), Podxl xKO with G-CSF (xKO-G-CSF). Bone marrow(BM) from the tibia and femur was collected. BM cells were flushed with HDM medium supplemented with 2% PBS and antibiotics and centrifuged. BM cells were passed through 251g syringe of 0.22 micron strainer. 0.5% Bovine Serum Albumin buffer and PBS, if 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 EasyStain Mouse Hematopoietic Progenitor Cell isolation kit according to manufacturer's protocol (BioLegend). Lineage negative population was obtained using immuno-magnetic separation. Number of lineage negative cells was increased. GMPs were blocked with rat and rabbit IgG and labeled by antibodies against CD34, CD15, CD16/32, CD41 and CD144 followed by fixation and permeabilization. Phospho-STAT3, STAT3, and CD11b antibodies were analyzed separately to tested groups and incubated for one hour. Samples were submitted to flow cytometry analysis. Flow cytometry data analysis was performed using FlowJo.

Figure 4: Loss of Podxl reduces activity of STAT3 during emergency granulopoiesis. The same experimental and data analysis approach and gating strategy was used as described in Figure 3. Anti-STAT3, Phospho-STAT3 PE and isotype control antibodies were also used. Statistical significance was performed using the Student's Two-Tailed T-Test for Significance. *p<0.05 ***p<0.001

Figure 5: Model of Podxl's role in G-CSF induced Emergency Granulopoiesis. Podxl is hypothesized to promote Jak3/STAT3 pathway. Jak3/STAT3 pathway consists of intracellular signaling cascades that control dendrimer-driven neutrophil production. Briefly, G-CSF phosphorylates transmembrane protein Jak2 which then phosphorylates STAT3. Newly activated STAT3 enters nucleus to activate transcription factors to promote reactive granulopoiesis, leading to production of neutrophils. Podcalyxin is in hematopoietic systems (right panel) leads to decreased GMPs production in bone marrow, with concomitant drop in mature neutrophils (data not shown). Loss of Podxl results in elevated expression of Jak3 and STAT3 on basal level and in emergency granulopoiesis. This activity is attributed to STAT3-dependent regulation of a feedback inhibition of G-CSF signal. STAT3 is a negative regulator of granulopoiesis but is not required for G-CSF-dependent differentiation. Immunity 2002;17(1):63-72

Discussion and Conclusions

We found that absence of Podxl in mice leads to upregulation of Jak3 activity and STAT3 in the myeloid lineage at the whole level and in G-CSF induced response. This negative effect of Podxl deletion on STAT3 phosphorylation coincided with markedly reduced neutrophil levels in GMPs. We found no difference in the total level of STAT3 in G-CSF-treated Podxl KO mice compared to wildtype mice, however total levels of STAT3 were increased in Podxl cKO in steady-state, suggesting an enhancement of regulatory feedback. Studies have shown that in steady state, STAT3 serves a negative regulatory function by suppressing accumulation of neutrophils in blood. This activity is attributed to STAT3-dependent regulation of a feedback inhibition of G-CSF signal. We recently found that Podxl deletion increases accumulation of the immature myeloid subpopulation with the associated drop in mature neutrophils in bone marrow, and increased levels of neutrophils in blood. We found that Podxl-deficient GMPs showed suppressed G-CSF-responsive growth relative to wild-type cells, however the underlying cellular response such as enhanced rate of differentiation of GMPs remained unclear. To conclude, our findings suggest that Podxl promotes the activation of STAT3 in the granulocytic lineage that directs G-CSF responsive myeloid progenitor proliferation.

In the future, we hope to explore the effects of the loss of Podxl on myeloid progenitors' self-cycle progression and maturation in response to G-CSF. We also hope to investigate if Podxl regulates neutrophil trafficking during emergency granulopoiesis.

**p<0.01 ***p<0.001

*Figures were collected using FlowJo. Statistical significance was performed using the Student's Two-Tailed T-Test for Significance. *p<0.05 ***p<0.001

**p<0.01 ***p<0.001

This work was supported by the SSRP program; thanks to Dr. Kozak, Dr. Liow and Liz Bergst. Thank you Dr. Sathyanarayana and Dr. Karaczyń for your guidance and support. WMEC 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, 1,416 women are expected to die from this disease in 2018.

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

**Methods**

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

**Exclusion Criteria**

National Cancer Database Ovarian cancer instances 2004-2015 (N=194,828)
- Non-invasive ovarian cancer (N=1,151)
- Invasive ovarian cancer (N=193,677)
- Patients treated in non-reporting facility (N=8,786)
- Patients missing stage (N=16,805)
- Patients missing travel distance (N=2,412)

**Final Cohort**

(N=165,674)

**Outcome Variable:**

**30-Day Mortality**

NCDB includes a binary variable, which tells whether a patient has died 30 days after their diagnosis.

**Study Variables**

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

**Covariates**

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

- **OUTCOME VARIABLE:**
  - 30 Day Mortality

**Results**

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

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

**Conclusion**

After controlling for covariates, we can say that, overall, travel distance does not have a statistically significant relationship with 30 day mortality among ovarian cancer patients. The next step, for a more in depth look at this question, would be to examine patients who travel further specifically to higher volume facilities in comparison to those who travel shorter distances to lower volume facilities and then to see if any effect is statistically and clinically significant.
Prowasan virus (Flaviviridae: Flavivirus) is a vector-borne disease that circulates in parts of North America and Russia in two serologically-indistinguishable lineages: lineage I (POWV) and lineage II (deer tick virus [DTV]). This study used 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.2  
- Exists primarily in a zoonotic cycle between ixodes scapularis ticks (deer ticks) and small-medium mammals (e.g. the white-footed mouse, woodchucks, and skunks).1  
- ~10% of POWV human infections result in deadly encephalitis7  
- 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.2  
- In the United States there has been a threefold increase in human infections in the past two decades (27 cases from 1958-1998 and 85 cases from 2003-2017), which may be due to rising deer tick populations in parts of North America.1  
- Ticks can be co-infected with other vector-borne diseases such as the agents of Lyme, anaplasmosis, babesiosis, and tick-borne relapsing fever.1

More southern counties in Maine reportedly have higher DTV infection rates compared with those in the north. This study was conducted in York County, which had an adult deer tick infection rate of 2.27% in 2017.8

Background

Objectives

- Investigate nidality by collecting ticks from five different habitat types and testing them for DTV to determine what constitutes a good pathobiocenose.9
- Determine what percentage of DTV-infected ticks were also infected with Lyme, anaplasmosis, babesiosis, and/or tick-borne relapsing fever.1

Hypotheses

- DTV-infected ticks will exhibit nidality (focality), meaning they will tend to exist within certain microhabitat-types more than others.1
- Co-infection will be observed in ticks infected with DTV.
Characterization of the Proteolytic Processing of CTHRC1

Kimberly L. Drew1, Qiaozeng Wang MS1, Yong-Ri Jin PhD1, and Volkhard Lindner MD,PhD1
1Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, Maine

Introduction:
Collagen triple helix repeat containing 1 (CTHRC1) is a hormone that is currently being researched as an endogenous factor in a variety of different health interests, including, but not limited to cancer, heart disease, inflammation, and estrogen signaling.1,2,3 As CTHRC1 is a versatile hormone, the proteolytic processing of this hormone, and where it is active and secreted, is an essential piece of information to use to characterize CTHRC1 further. The understanding of its role in these complex medical interests, the proteolytic processing of CTHRC1, is not clearly understood, specifically whether or not the hormone needs to be activated and if so how it is activated and secreted. CTHRC1 is expressed in neoplastic cells, which require regulated secretion to be activated and this further to conclusions that CTHRC1 might be processed in a regulated and secreted secretion as well. As CTHRC1 has a terminal lysine, therefore in the CTHRC processing pathway, where the tRNA translational modification occurs outside of the cell and is a large part of CPE, which plays a large role in the proteolytic processing of CTHRC1. To characterize this possible mechanism, transfected 293-T cells that were plasmid-transfected and indicated plasmid were collected as samples of conditioned medium (CM) and cell lysate as well as the conditioned medium and cell lysate were immunoblotted to observe the translational modification of CTHRC1's maturation steps.

Results:

1. Figure 1: Schematic of the KIMC processing pathway in transfected 293-T cells.

2. Figure 2: Western blotting of transfected 293-T cells with four plasmid constructs hFL, hFLΔK, VVD, and -502mychis (rat CTHRC1 with myc tag).

3. Figure 3: transfected 293-T cells per RIMC in the cell lysate and does not secrete into the conditioned medium.

4. Figure 4: Transfection of 293-T cells with plasmid construct CMV-EGFP as control was transfected with four plasmid CTHRC1 constructs.

5. Figure 5: Western blotting of transfected 293-T cells with plasmid construct CMV-EGFP as control was transfected with four plasmid CTHRC1 constructs.

6. Figure 6: Transfection of 293-T cells with plasmid construct CMV-EGFP as control was transfected with four plasmid CTHRC1 constructs.

7. Figure 7: Cell lysate of transfected 293-T cells collected and placed on gel do not appear to display translation modification.

8. Figure 8: Transfected 293-T cells co-expressed with CPE show no evidence of secretion into the conditioned medium.

9. Figure 9: Transfected 293-T cells co-expressed with CPE show no evidence of secretion into the conditioned medium.

10. Figure 10: Transfected 293-T cells co-expressed with CPE show no evidence of secretion into the conditioned medium.

11. Figure 11: Cell lysate of transfected 293-T cells with plasmid construct CMV-EGFP as control was transfected with four plasmid CTHRC1 constructs.

Summary and Conclusions:
Results:

1. 293-T cells cannot cleave CTHRC1 efficiently, even with the addition of CPE.

2. In the cell lysates, the lower, denser band is the non- glycosylated version, whereas the higher band is the glycosylated version.

3. In the AtT20 and CHO cells transfected with hFL and FDLK, there was no indication of a higher band in the conditioned medium of AtT20 and FDLK, then that of the cell lysate. This indicates a further post-translational modification that occurs outside of the cell with these plasmids.

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

5. In the AtT20 cell transfection Western Blot there is no distinction between the cell especially with the difficulties in reading the Western Blot due to the non-specific bands of a single site, which was unexpected. Assuming that there were no difficulties with the transcription that indicates that this construct is only minimally able to be secreted.

6. Posttranslational mass spectrometry determined that CHO cells CM have its terminal lysine, and so the result of CTHRC1 not having expressed endogenous CPE does not disprove the hypothesis.

7. Neither the CHO transfection, samples transfected with 502mychis, show no shift between the CM and CL. This may be determined by the c-terminal tag having influencing the post-translational modifications that take place outside of the cell.

8. Along with the observation, the CM LysC Western Blot that includes the expression of CTHRC1. With the CM in 293-T cells may indicate that the c- terminal lysine remains with the ability for 293-T cells to secrete CTHRC1 efficiently.

9. In the 293 cells when co-transfected with CPE+FLDKE, the top band is higher than the non-CPE and CPE and appears to be a timer, which is unlikely after the labeling involved in Western Blot. This observation does not have a conclusion aside from the possibility that the top band is higher than the non-CPE, and will continue to be explored in future experiments.

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

Acknowledgements:
The work in this project was supported possible without the support of the members of the Linde Lab - Volkhard Lindner MD, Ph.D., Joseph Woodard, JN. PhD, Arna Maringbo, and Mayasah Al Hashimi, and many of the faculty of MMCRI with all of their support, expertise, and patience. The funding for this research was provided by the National Heart, Lung, and Blood Institute Summer Student Research Program and the American Heart Association. Thank you so much for this opportunity!

References:

1. et al.: 2018 MMCRI Posters

2. Snyder, M. F., & Chait, B. T. (2009). An endogenous hormone of the steroid hormone superfamily encoded within the human genome. Cell, 139(4), 801-810.

3. Cawley, N. X., Wetsel, W. C., Murthy, S. R. K., Park, J. J., Pacak, K., & Loh, Y. P. (2008). Carcinoid tumor-derived JIMC is a novel hormone that interacts with the CTHRC1 signaling pathways. Journal of Clinical Investigation, 118(11), 3115-3126.

4. Adarichev, V. I., & Blel, J. (2010). Effects of structural modifications on the activity and stability of a new class of proteins. FEBS Letters, 584(11), 1827-1831.

5. Pfeifer, S. D., & Adarichev, V. I. (2013). Structural modifications of human CTHRC1 and JIMC: A novel class of proteins. FEBS Letters, 587(3), 517-522.

6. Cawley, N. X., Wetsel, W. C., & Loh, Y. P. (2009). Carcinoid tumor-derived JIMC is a novel hormone that interacts with the CTHRC1 signaling pathways. Journal of Clinical Investigation, 119(11), 3533-3540.

7. Cawley, N. X., Wetsel, W. C., & Loh, Y. P. (2008). Carcinoid tumor-derived JIMC is a novel hormone that interacts with the CTHRC1 signaling pathways. Journal of Clinical Investigation, 118(11), 3115-3126.

8. Adarichev, V. I., & Blel, J. (2010). Effects of structural modifications on the activity and stability of a new class of proteins. FEBS Letters, 584(11), 1827-1831.

9. Pfeifer, S. D., & Adarichev, V. I. (2013). Structural modifications of human CTHRC1 and JIMC: A novel class of proteins. FEBS Letters, 587(3), 517-522.

10. Cawley, N. X., Wetsel, W. C., Murthy, S. R. K., Park, J. J., Pacak, K., & Loh, Y. P. (2008). Carcinoid tumor-derived JIMC is a novel hormone that interacts with the CTHRC1 signaling pathways. Journal of Clinical Investigation, 118(11), 3115-3126.
Results

Children and ASD seeking care in inpatient psychiatric units could benefit greatly from genetic testing due to the complex natures of their diagnoses and behavior, which often include lower verbal levels, intellectual disability, self-injurious behavior and emotional dysregulation.  

Objectives

To examine the prevalence and findings of genetic testing in an inpatient community of children diagnosed with ASD.

Methods

936 participants with ASD confirmed by direct diagnosis using the Autism Diagnostic Observation Schedule (ADOS) were recruited from the Autism and Developmental Disorders Inpatient Research Collaborative (ADDIRC). A study conducted in 6 child psychiatric units in the US specializing in treatment of ASD.

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

| Demographic                  | Genetic Testing (N = 729) | No Genetic Testing (N = 107) | p Value |
|------------------------------|---------------------------|-------------------------------|---------|
| Age (Years) (ADHD)           | 13.32 (5.45)              | 12.4 (3.34)                   | 0.024   |
| Sex (Male) (N%)              | 238 (75.7%)               | 150 (44.5%)                   | 0.004   |
| ADOS Module (N%)             |                           |                               |         |
| 1                           | 141 (45.5%)               | 125 (35.5%)                   | 0.218   |
| 2                           | 44 (13.5%)                | 40 (11.6%)                    | 0.683   |
| 3                           | 31 (9.6%)                 | 39 (11.2%)                    | 0.039   |
| Parental Education (N%)      |                           |                               |         |
| Less Than High School       | 238 (75.7%)               | 150 (44.5%)                   | 0.004   |
| Adopted (N%)                | 52 (16.1%)                | 46 (13.4%)                    | 0.586   |
| Household Income             |                           |                               |         |
| Less than $80,000            | 186 (62.6%)               | 121 (34.5%)                   | 0.000   |
| $80,000 - $160,000           | 77 (25.9%)                | 94 (26.9%)                    | 0.778   |
| More than $160,000           | 66 (21.4%)                | 44 (12.9%)                    | 0.000   |

• Of the total child sample, 588 (80.6%) were male and the average age was 12.50 (SD = 3.33).
• 436 (54.3%) children were non- or minimally-verbal (determined by ADOS Module administered).
• There were statistically significant differences in gender, ADOS module, and household income between the two genetic testing groups.
• Those with genetic testing were more likely to be non- or minimally-verbal, and have a household income of more than $160,000.

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

43.07% of parents reported having genetic testing for their child prior to their admission.

Bivariate logistic regressions were conducted to examine the of parent reported genetic testing in hospitalized children with ASD.

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

69.66% of parents with a significant result reported that genetic testing resulted in some "other" finding and 15.7% reported a Fragile X diagnosis.

Of the Other results, 9.68% were disorders known to have ASD etiology and 8.06% were considered ASD candidate genes.

Figure 3A-C: Health, Behavioral, and Demographic Influences on Genetic Testing

A

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

B

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

Conclusions

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

References

1. Bache, R. A., & Buxton, L. (2012). Access to genetic services and the genetic health of families with a child with autism spectrum disorders: 2013 guideline revisions. Genetics in medicine, 15(4), 399–407.
2. Siegel, L. M., & Duyme, L. (2017). The American College of Medical Genetics and Genomics’ Standards and Practices for the Provision of Genetic Services. Genetics in medicine, 19(10), 1059–1071.
3. Schellekens, S. R., & Buxton, L. (2015). Parental access to genetic testing and the genetic health of children with autism spectrum disorders: A systematic review and meta-analysis. Journal of Child Psychology and Psychiatry, 56(6), 598–608.
4. Van Vliet, J. N., Van der Meere, J., & Leventhal, A. (2013). Fragile X syndrome: diagnosis and management. Pediatr Rev, 34(1), 10–15.
5. gene lists for the primary disorders. J. Consult. Clin. Psychol. 84(5), 797–807.
6. Schellekens, S. R., & Buxton, L. (2015). Fragment analysis of FMR1 in the general population: the FMR1 gene list. Journal of Medical Genetics, 52(9), 550–555.

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 uncertain significance, its clinical value is currently unproven.
• When using GTT, physicians need to counsel patients about both its value and its limitations, but the ideal goals and elements of physician-patient discussions have not been clearly defined.
• 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™

Key Goals of Discussions
“What do you think are the three (3) most important goals when introducing genomic tumor testing?”

| Goal Options | n |
|--------------|---|
| Inform patients about benefits and limitations of genomic tumor testing | 66 |
| Manage patients expectations about genomic tumor testing | 56 |
| Facilitate decision making | 54 |
| Understand patient preferences for genomic tumor testing | 30 |
| Maintain patient hope | 9 |
| Encourage patients to undergo genomic tumor testing | 3 |
| Share your personal viewpoints on genomic tumor testing | 1 |

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

| Nature of GTT (n=201) | Uncertainty about GTT (n=59) | Potential Outcomes of GTT (n=25) | Uncertainty about Therapeutic Options (n=26) | Potential Harms (n=7) |
|---------------------|---------------------------|-----------------------------|----------------------------------|------------------|
| Logistics of Testing (n=43) | Therapeutic Implications (n=43) | Genetics & Genomics (n=21) | Discovery of Germline Variants (n=11) | Patient Expectations (n=24) |

Illustrative Quotations from Open-ended Responses

| Discuss nature of GTT | Convey uncertainty about GTT | Maintain expectations about GTT |
|-----------------------|-------------------------------|---------------------------------|
| “Every cancer is unique / This is a way to utilize precision medicine and offer a more personalized treatment based on cancer genomics and identifying specific genes and mutations associated with patient’s individual cancer” | “The chance of finding a practical – treatment is small but if found could lead to major benefit” | “The results may help identify one, none, or several mutations in your tumor / we hope the results will help guide future treatment options, however we don’t always find mutations that we can take action on- or change the treatment” |

Conclusions
• Cancer care providers identify several different goals for physician-patient discussions about GTT.
  • The most commonly prioritized goals relate to informed and shared decision making, and managing patient expectations.
  • Providers identify a variety of different content elements for physician-patient discussions about GTT.
  • Key elements regarding the nature of GTT focus mainly on the meaning and rationale for GTT, rather than its potential harms or disadvantages.
  • Key elements regarding uncertainty in GTT focus mainly on therapeutic options and incomplete evidence.
• Future research directions:
  • Replicate and assess the generalizability of the findings in a larger, more diverse sample.
  • Assess patients’ perceptions of the goals and ideal content of physician-patient discussions of GTT.
  • Develop and test patient education and decision support interventions to facilitate informed and shared decision making about GTT.
  • Develop and test interventions to train physicians in facilitating shared decision making and managing patient expectations about GTT.

References
• Peterson, E. B., et al. (2016). “Communication of cancer-related genetic and genomic information: A landscape analysis of reviews.” Transl Behav Med 6(1): 59-70.

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

a.mccown207@gmail.com

Image: Maine Cancer Genomics Initiative, Maine Medical Center Research Institute (MMCRI)
Flow Cytometric Analysis of Circulating Microparticles After Cardiac Arrest

Nathan L. Pinnett1, Mary Weatherbee1, Joanne Dekay1, M.S., Sarah Peterson1, M.D., PhD, Amanda Kost1, B.S., Hai fung Yin1, PhD, Douglas Sawyer1, 2, M.D., PhD, Michael Robich1, 2, M.D., David Seder2, MD, Sergey Ryzhov, 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 (arythmia). 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® tubes. Platelet-free plasma was prepared at room temperature using two-step centrifugation, each at 2,000 rpm for 20 minutes. After preparation plasma was stored at -80°C until further analysis.

Cerebral Performance Category (CPC): CPC is a neurological test based on a scale of 1 through 5. 1 being the best and 5 being the worst.

- 1 return to normal cerebral function and normal living
- 2 - cerebral disability but sufficient function for independent activities of daily living
- 3 - severe disability, limited cognition, inability to carry out independent existence
- 4 - coma
- 5 - brain death

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

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

RESULTS

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

Figure 2. The number of circulating microparticles in CA subjects at 6 hours is significantly higher compared at 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.
Sympathetic and sensory innervation of bone modulates remodeling
Nick Banks, Audrie Langlais BS; Audrey Bergeron MS; Roni Kunst MS; Adriana Leilis Carvalho (PhD), K. Motyl, (PhD)
Center for Molecular Medicine (CMM), Maine Medical Center Research Institute (MMCRI), Scarborough, ME, US.

Abstract
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 to the story (Figure 2). The precise mechanisms of neuro-osteogenic crosstalk remain obscured. A better understanding innervation with respect to bone remodeling will inform treatment of bone pathologies and precise mechanisms of neuroskeletal interactions. One of the biggest challenges is to visualize sensory nerves as they innervate bone. New methods of visualizing sensory nerves have been developed recently that utilize quantum dots. These 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, growth factors, 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 antigenic sections of long bones and L5 vertebrae (Figure 1) conjugated to secondary antibodies (Figure 3) to compensate for signal amplification; for signal amplification to compensate for this fixation time and decalcification reduces antigenicity. This diminishes signal strength, which in turn decreases imaging signal-to-noise ratio. This makes it difficult to image sensory nerves if they are not properly stained. New methods for visualizing sensory nerves have been developed recently that utilize quantum dots. Quantum dots (Figure 4) have more desirable optical qualities than organic dyes and fluorophores, and will be used to overcome the inherent difficulty in confocal imaging of bone.

Methods and Materials
• Fixed, frozen sections of long bones and LS vertebrae from transgenic mice expressing GFP driven by sequence under trpm8 promoter.
• IHC, performed to visualize neural markers and trpm8 fluorescent markers. (Figure 5)
• Quantum dots (Figure 6) conjugated to secondary antibodies (Figure 7) in lieu of organic fluorophores, for signal amplification to compensate for high auto fluorescence and as proof of principle for future studies.
• Images (Figures 6, 7) from confocal microscope analyzed with Autopaint and Imaris software.

Results
• Strong evidence supporting sensory innervation along periosteum of femur. TrkA Antibody Staining (Figure 8).
• Afferent sensory nerves relay local mechanical conditions to the brain (Figure 1).

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 line” morphological characteristics. Must tailor to detergent concentration, antibody type/dilution/Incubation times to optimize staining. Rigorous negative controls and positive controls (both dorsal root ganglia) will be required to confirm specificity of assay results. Further investigation of trpm8 localization in bone needed to reveal any potential role in mediating bone homeostasis.

Future Directions
•Multiplex imaging (Figure 8) of multiple neural markers and receptor (TH, CGRP, TRPM8) to show colocalization, grom due to communication mechanisms.
• Quantitative comparison of neural organization and density between long bones and vertebrae.
• Study changes in neural density from neuropathy in mouse B10 DBA/OIb diabetes model.

Acknowledgments: This research is supported by NIH/NIAMS P01GM59278 to K.J.M, NIH/NIAMS P30GM103418 to K.J.M, NIH/NIAMS P30GM103418, P30GM103418, P01GM121301 US4GM11516 to Maine Medical Center.
Contact: Katherine J. Motyl; 81 Research Dr. Scarborough, ME 04074; kmotyl@mmcri.org.

References
1. Charter, S.; Thompson, M.; Lodige, G.; Prud, M.; Males, L. and Motyl, K. (2016). Evident sprouting of sensory and sympathetic nerve fibers in mouse bone fractures and the prevention and maintenance of chronic osteoporosis. J. Bone Miner. Metab., 34(3), 242-256.
2. Kitayama, M., Nakamura, S., Nishikawa, M., and Motyl, K. (2015). Expression and localization of neurokinin-1 mRNA in the developing mouse spine. J. Neurosci., 35(23), 9161-9166.
3. Kitayama, M., Nakamura, S., Nishikawa, M., and Motyl, K. (2015). Sample preparation for high-resolution 3D confocal imaging of mouse dissected spine. Nature Protocols, 10(12), 2385-2414.
4. Motyl, K. and Lelis Carvalho, A. (2014). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
5. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
6. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
7. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
8. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
9. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
10. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
11. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
12. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
13. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
14. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
15. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
16. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
17. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
18. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
19. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
20. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
21. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
22. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
23. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
24. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
25. Motyl, K. and Scarbourough, W. (2013). The Baroreflex and the Sympathetic Nervous System: it’s about Time. The Journal of Clinical Endocrinology & Metabolism, 91(11), pp. 4069-4071.
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 vasculogenesis 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 gonad al 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

| Gene | Name | IWAT CD | IWAT HFD | BAT CD | BAT HFD | PVAT CD | PVAT HFD |
|------|------|---------|----------|--------|---------|---------|----------|
| TAGL2 | Very long chain acyl-CoA synthase | 1,041 | 6,294 | 19,618 | 33,436 | 2,436 | 16,485 |
| HSDL2 | Hydroxysteroid dehydrogenase-like protein 2 | 6,773 | 50,774 | 2,399 | 6,749 | 2,115 | 11,594 |
| FUS1 | Perilipin-2 | 16,034 | 219,927 | 19,137 | 33,800 | 41,056 | 475,215 |
| ANXA1 | Annexin A1 | 1,311,051 | 2,012,222 | 84,678 | 138,127 | 49,419 | 111,520 |
| HMCS2 | Hydroxymethylglutaryl-CoA synthase | 2,436 | 2,115 | 200% | 150% |
| S27A2 | Hydroxysteroid dehydrogenase-like protein 2 | 142,657 | 156,959 | 317,538 | 475,215 | 136,110 | 309,144 |
| TAGL2 | Transgelin-2 | 773,866 | 1,083,330 | 198,420 | 293,258 | 123,724 | 196,127 |

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

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

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

### 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 Karolak (MMCR Molecular Phenotyping Core Manager) for maintaining the 384 Thermocycler with which I have become intimately familiar.

https://knowledgeconnection.mainehealth.org/jmmc/vol1/iss1/16

DOI: 10.46804/2641-2225.1017

20
Pediatric Interfacility Transfers – Association of Pre-transfer Vital Signs with Length of Stay at a Tertiary Care Center
Sarah Bunting BA, Leah Mallory MD, Logan Murray MD
Maine Medical Center, Department of Pediatrics, Portland Maine

Background

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

Objectives

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

Materials and Methods

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

Statistical analyses were performed using SPSS™ statistical software, version 25 (IBM SPSS Inc, Armonk, NY).

References

1) Rosenfeld JL, Hilton JJ, Tesfai BJ, 2nd, Ramani PS, Kruger AJ, Okumura MJ. Profiling Interfacility Transfers for Hospitalized Pediatric Patients. Hosp Pediatr. 2016;6(6):345-353.
2) Gattu RK, De Foe AS, Lichenstein R, Teshome G. Consideration of Cost of Care in Pediatric Interfacility Transfers: A Case Study of the American Academy of Pediatrics; 2016.
3) Simon TD, Cawthon ML, Stanford S, et al. Pediatric medical complexity algorithm: a new tool to stratify children by medical complexity. Pediatr Med. 2014;133(s):1647-1654.
4) Pediatric advanced life support: Provider Manual. Dallas, TX: American Heart Association, American Academy of Pediatrics, 2016.

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 < 0.001). 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 10.75 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 decompensation such as Bedside Pediatric Early Warning System (BPEWS) with LOS.

Acknowledgments

Special thanks to Wendy Y. Craig, PhD at the Maine Medical Center Research Institute for her invaluable assistance with statistical analysis of data which was supported in part by the Northern New England Clinical and Translational Research grant U54GM115516.
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. Pervascular adipose tissue (PVAT) surrounds most of the large blood vessels including the aorta. PVAT has thermogenic and vaso-protective properties. In obesity PVAT exhibits structural and functional changes. Unhealthy adipose tissue can become inflamed and inhibit the beneficial adipokines. Methionine reduction has shown to increase health span in mouse models. The methionine restricted mice continued to maintain healthy weight and improved glucose metabolism. To understand how methionine restriction alters adipose tissues, we initially used the 3T3-L1 cell line. 3T3-L1 is a fibroblastic line that can be differentiated into adipocytes and will be a useful model for altering methionine during adipogenesis in vitro. We used an adipogenic cocktail to induce these cells along the path to mature adipocytes. Oil Red O staining was used to view lipid accumulation and we performed initial studies with different concentrations of methionine in the medium. Our next aim is to test if the D-isomer of methionine is metabolized differently than the L-isomer. This is important to develop an accurate optimal methionine concentration. Having a cell model will be beneficial for future study into activities of methionine restriction in adipocytes. In addition, it will set the stage for continued studies with primary cells derived from PVAT.

Materials and Methods

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

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

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

Results

3T3-L1 model of adipocyte differentiation

![Image](image1)

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.

![Image](image2)

Figure 2. 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 model to test the effects of methionine restriction on the adipogenic capacity of the cells, we found that cells containing a racemic mixture of 0.0184mM methionine (D and L isomers) had enhanced

Acknowledgements and sources of support

Research reported in this project was supported by an Institutional Development Award (IDeA) grant number P20GM103423.

from the National Institute of General Medical Sciences.
Characterizing the pluripotency of human thoracic perivascular adipose tissue progenitor cells

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

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

Abstract

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

Background

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

Adipogenic

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

Osteogenic

Staining of the bone marrow MSCs showed a markedly larger amount of mineralization than the PVAT cells, in which the staining was sparse. This suggests that the human thoracic PVAT cells were not able to differentiate toward an osteogenic lineage by the 14 day time point, unlike the MSCs.

Chondrogenic

Fig. 3. In the chondrogenic induction assay, shown in these images, human thoracic PVAT cells were cultured using a “micromass” technique which allows for the growth of a pellet of roughly 10^6 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,
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 20181.
• The only curative treatment at this time is resection, yet only 10-20% of patients are considered clinically resectable at the time of presentation2.
• 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 studies3.
• Studies have shown that receiving chemotherapy at some point is advantageous compared to just surgery, but the sequence is debated4.

Objectives

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

Materials and Methods

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

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

References

1. Cronin KA, Lake AJ, Scott S, et al. Annual Report to the Nation on the Status of Cancer, part 1: National cancer statistics. Cancer. 2018;124(13):2785-2800.
2. Wagner M, Redaelli C, Lietz M, Seiler CA, Friess H, Buchler MW. Curative resection is the single most important factor determining outcome in patients with pancreatic adenocarcinoma. Br J Surg. 2004;91(5):586-594.
3. de Geus SW, Evans DB, Bliss LA, et al. Neoadjuvant therapy versus upfront surgical strategies in resectable pancreatic cancer: A Markov decision analysis. Eur J Surg Oncol. 2016;42(10):1552-1560.
4. Epelboym I, Zenati MS, Hamad A, et al. Analysis of Perioperative Chemotherapy in Resected Pancreatic Cancer: Identifying the Number and Sequence of Chemotherapy Cycles Needed to Optimize Survival. Ann Surg Oncol. 2017;24(9):2744-2751.

| Variable          | HR (95% CI) | P value |
|-------------------|-------------|---------|
| Age, 65-79        | 1.15 (1.09-1.21) | <0.0001 |
| Age, 80-89        | 1.62 (1.56-1.77) | <0.0001 |
| Male              | 1.05 (1.01-1.09) | 0.004   |
| White             | 1.02 (0.97-1.06) | 0.83    |
| Medicare          | 1.02 (0.96-1.08) | 0.955   |
| Private insurance | 0.88 (0.83-0.93) | <0.0001 |
| Academic center   | 0.79 (0.77-0.82) | <0.0001 |
| Metropolitan area | 0.92 (0.89-0.95) | <0.0001 |
| Comorbidities     | 1.33 (1.23-1.45) | <0.0001 |
| Poor tumor grade  | 1.23 (1.21-1.35) | <0.0001 |
| Head/Neck Tumor   | 1.09 (1.06-1.13) | <0.0001 |
| Start with Surgery| 0.48 (0.46-0.51) | <0.0001 |

Next Steps

• 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.
• Prospective, randomized controlled trials comparing neoadjuvant therapy against an upfront surgical approach are needed to better answer this question.
INFLUENCE OF LYSOSOMAL ACID LIPASE ON OSTEOBLAST DIFFERENTIATION AND FUNCTION

Talia Staiger¹, Elizabeth Rendina-Ruedy¹, Ron C. Helderman¹, Liv Palma¹, and Clifford J. Rosen¹
¹Maine Medical Center Research Institute, Scarborough, ME, 04074

INTRODUCTION

- Lysosomal acid lipase (LAL) is an enzyme in the lysosome that breaks down cholesteryl esters (CE) and triacylglycerides (TAGs).¹
- In humans, an LAL deficiency presents itself in two diseases:
  - Wolman Disease - infants experience severe hepatosplenomegaly and malabsorption and usually die before one year of age
  - Cholesterol ester storage disease - later onset and milder phenotype, resulting in TAG and CE storage in various tissues ²
- LAL knockout (KO) mice have increased TAG and CE storage in various tissues ³, as well as defective brown adipose tissue, leading to hypothermia at room temperature.⁴
- Recently, our lab has demonstrated LAL KO mice also have decreased bone volume/total volume (BV/TV) in the distal femur metaphysis.

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 (BMSC) were treated with 3 mL of trypsin for three minutes before 20 mL alpha MEM complete medium was added. The cells were counted and plated at a concentration of 5.0 x 10⁵ cells per well in three 12-well plates.

Lalistat Treatment

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

RESULTS

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

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

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

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

REFERENCES

1. Du, H. (1998). Targeted disruption of the mouse lysosomal acid lipase gene: long-term survival with massive cholesteryl ester and triglyceride storage. Human Molecular Genetics, Vol. 7.
2. Du, H. (2001). Lysosomal acid lipase deficient mice: depletion of white and brown fat, severe hepatosplenomegaly, and shortened life span. Journal of Lipid Research, Vol. 42.
3. Duta-Mare, M. (2018). Lysosomal acid lipase regulates fatty acid channeling in brown adipose tissue to maintain thermogenesis. BBA - Molecular and Cell Biology of Lipids.

ACKNOWLEDGEMENTS

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

CONCLUSIONS & SUMMARY

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

HYPOTHESIS AND EXPECTED OUTCOMES

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

Schematic Depicting LAL’s Role

“Normal” LAL

LAL Knockout (KO) Mice

Angiogenesis

Bone Marrow Stromal (BMSC) Cell Culture

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

CONCLUSIONS & SUMMARY

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

ACKNOWLEDGEMENTS

This internship was supported by the Summer Student Research Program at Maine Medical Center Research Institute. I would also like to acknowledge all of the members of the Rosen lab, Liz Berger, Drs. Lucy Linne and Rob Kona.
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. 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. The goal of this study was to examine data from the pupillometer (specifically Neurological Pupil Index (NPi), percent constriction, constriction velocity, and pupil size) and compare the values to previously-established thresholds 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.

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

Conclusions

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

References

1) Shyombo et al. Understanding the Relationship Between the Neurological Pupil Index and Constriction Velocity. Scientific Reports. 2018:8(6992):1-6.
2) Eikel et al. Early Prediction of Coma Recovery after Cardiac Arrest using Pupilometry. American Neurological Association. 2017:0(0):1-7.
Atrial fibrillation (AF) is the most prevalent and frequently encountered heart arrhythmia in adults, especially within the emergency department (ED).

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

However, when used electrical cardioversion has the highest overall success rate for patients presenting with atrial fibrillation. Utilizing electrical cardioversion as a primary treatment method for atrial fibrillation will lead to faster patient turnarounds within the emergency department.
Abstract
Accumulation of excess fat in white adipose tissue (WAT) is associated with an increase in risk for type 2 diabetes (T2D). Unlike brown adipose tissue (BAT), burns calories by releasing energy stored in fats to generate heat. This process, termed thermogenesis, makes BAT critical for reducing obesity risks, especially 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 BAT derived exosomes can 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.

Hypothesis
We hypothesized that activated human brown adipocytes grown in cell culture can be used to isolate exosomes to determine microRNA content that may alter gene expression in an anti-diabetic manner in distant tissues. We found that this microRNA-32 is enriched in exosomes compared to brown adipocytes and miRNA expression was previously shown to be expressed by brown adipocytes, miRNA-32 expression was previously shown to be necessary for activation of brown adipocytes (Cell Rep. 2017; 19(6):1229-1246). We found that this miRNA-32 is enriched in exosomes (>85 fold) compared to brown adipocytes from which these secreted factors act on distant tissues. We found that this miRNA-32 is enriched in exosomes (>85 fold) compared to brown adipocytes from which these secreted factors act on distant tissues. 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.

Methods
1) Grow brown adipocytes in culture.
2) Isolate exosomes from conditioned medium.
3) Purify microRNAs 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
Brown adipocytes
Isolation of exosomes from brown adipocytes
Exosomes were isolated from conditioned medium (3 days conditioning) using the Total Exosome Isolation Reagent purchased from Thermofisher Scientific. Briefly 4 ml of conditioned medium was spun by centrifugation at 10,000g for 1 hour, and the resulting exosome pellet was re-suspended in PBS. Flow cytometry was used to determine the presence of exosomes after staining for 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 adipocyte exosomes showed a gel band in the approximate range expected for miRNAs (25-200 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 transcriptase primers for 3 exosomal microRNA markers previously shown to be expressed by brown adipocytes, miRNA-32, miRNA-99b and U6, the U6 snRNA is a non-coding small nuclear RNA commonly used as internal control to normalize miRNA expression in cells. cDNA was amplified by qPCR using TaqMan probe based assays for these 3 targets. These data suggest that we were able to successfully isolate microRNA from the exosomes of brown adipocytes.

Acknowledgements
This work was supported by NIH COBRE award P20GM121301 (A. Brown, L. Liow, 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. Frissell, PI).

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

https://knowledgeconnection.mainehealth.org/jmmc/vol1/iss1/16
DOI: 10.46804/2641-2225.1017
28
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 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). Eto (Eto) is an pharmacological irreversible inhibitor of CPT1, effectively inhibiting fatty acid oxidation. In other cancers, such as breast and prostate cancer, inhibiting fatty acid oxidation with the use of etomoxir has been proven to reduce cancer cell viability and proliferation. Recently etomoxir has been shown to have off target effects by inhibiting complex one of the electron transport chain at high dosages (4).

In addition to a potential energy source, BMAT has been shown to increase MM’s resistance to chemotherapeutic treatments (5). We are examining the effect of etomoxir on different MM cell lines and if it increases MM sensitivity to chemotherapeutic drugs.

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

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

METHODS

Cell Culture: MM1S (gfp+/luc+), MM1R (gfp+/luc+), and OPM2 (mcherry/luc+) were seeded at various densities into clear and white 96 well plates.

Drug administration: Etomoxir (CPT1 inhibitor), Bortezomib (proteasome inhibitor), and Dexamethasone (corticosteroid that reduces inflammation) were added at various doses 24 hours after the cells were seeded.

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.

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

RESULTS

2. In vitro Combination Treatment of Etomoxir and Bortezomib

A OPM2: Etomoxir + Bortezomib

B MM1R: Etomoxir + Bortezomib

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

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

MM1S cells were directly co-cultured with mouse mesenchymal stem cells (mMSCs) for 24 hours before etomoxir (5µM) and dexamethasone (0.5µM) were administered. The mMSCs were seeded at a cell density of 7500 cells/well. The MM1S cells were seeded at a cell density of 5000...