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

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

The following posters were presented as part of the 2018 MMCRI Summer Student Research Program. This program offers undergraduates and medical students a unique opportunity to conduct research in diverse clinical and biomedical science fields during the summer months. During the paid ten-week program, students participate in mentored independent research projects either in our state-of-the-art research facility, or working with physicians in a hospital setting to impact patient care or the outcome of treatment. Students also attend lectures and workshops featuring topics including bioethics, animal use in biomedical science and scientific presentation skills, and have the opportunity to attend presentations by guest scientists and MMCRI faculty. All students give a final presentation, which in 2018 involved a three minute oral presentation called a “Three Minute Thesis” as well as a scientific poster presentation. All authors have an affiliation with MMCRI, unless otherwise noted.

The Role of Lipid Metabolism in Multiple Myeloma

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INTRODUCTION

- **Multiple Myeloma (MM)** is the second most common type of hematological cancer, formed from a series of oncogenic mutations to the plasma cells of the bone marrow (1).
- Initially patients respond well to chemotherapeutic treatment, but almost all eventually develop resistance to these treatments and experience relapse.
- Myeloma thrives in the unique and complex bone marrow microenvironment. Also, within the bone marrow are bone marrow adipocytes (BMA) that form bone marrow adipose tissue and account for 50-70% of the total bone marrow volume. It is believed that BMAT provides a source of energy that aids in multiple myeloma cell metastasis (2).
- Fatty acid oxidation is the process by which cells convert long chain fatty acids into NADH, FADH₂, and ATP in the mitochondria.
- CPT1 is a transport enzyme in the outer mitochondrial membrane that transports long chain fatty acids into the inner mitochondrial space. It is the first, yet rate limiting enzyme of the carnitine system and subsequently of fatty acid oxidation (3).
- Etomoxir (Eto) is a pharmacological irreversible inhibitor of CPT1, effectively inhibiting fatty acid oxidation.
- In other cancers, such as breast and prostate cancer, inhibiting fatty acid oxidation with the use of etomoxir has been proven to reduce cancer cell viability and proliferation.
- Recently etomoxir has been shown to have off target effects by inhibiting 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. Bortezomib and dexamethasone are common pharmacological anti-myeloma treatments.

Cell Number: Bioluminescent imaging (BLI) was used to quantify cell viability. Luciferin (10 μ L) 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

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

CONCLUSIONS

1. We have seen a decrease in cell viability in our *in vitro* models with the MM1S, MM1R, and OPM2 multiple myeloma cell lines when treated with etomoxir only.
2. Our preliminary data showing that etomoxir decreased cell viability, suggests that multiple myeloma may utilize fatty acid metabolism as a source of energy.
3. Combination treatments of etomoxir with other anti-myeloma drugs, such as bortezomib and dexamethasone showed a significant decrease in cell viability when compared to the control in MM1S and MM1R cell lines.
4. While we are seeing a trend in a reduction of cell viability with the use of etomoxir in all cell lines, more research is required to confirm this data and refute any claims of off target effects.

FUTURE DIRECTIONS

1. Isolate mitochondria to run a SeaHorse assay to determine the dose of etomoxir that results in off target effects on the electron transport chain.
2. Determine the exact doses of etomoxir in combination with bortezomib and dexamethasone that are most effective in multiple myeloma cell lines.
3. Seed myeloma cells in direct, or indirect, co-culture with bone marrow adipocytes to investigate whether etomoxir has a greater negative effect on cell viability in a high lipid environment.
4. Create a CPT1 knockdown model using lipofectamine and siRNA to see the effect of complete inhibition of fatty acid oxidation without the risk of off-target effects.
5. Treat multiple myeloma cells with a combination of etomoxir and orlistat (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, orlistat, and anti-myeloma drugs.

REFERENCES

1. Kyle, R. A., & Rajkumar, S. V. (2008). Multiple myeloma. *Blood*, 111(6), 2962–2972.
2. Fazeli, P. K. et al. (2013). Marrow Fat and Bone—New Perspectives. *J. Clin. Endocrinol. Metab.* 98, 935–945.
3. Gatz, M. L., Silva, G. O., Parker, J. S., Fan, C., & Perou, C. M. (2014). An integrated genomics approach identifies drivers of proliferation in luminal-subtype human breast cancer. *Nature Genetics*, 46(10), 1051–1059. DOI: 10.1038/ng.3073
4. Yao CH, Liu GY, Wang R, Moon SH, Gross RW, et al. (2018) Identifying off-target effects of etomoxir reveals that carnitine palmitoyltransferase I is essential for cancer cell proliferation independent of β -oxidation. *PLOS Biology* 16(3): e2003782.
5. Falank, C., Fairfield, 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. B., Valentino, A., Margarucci, 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.

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

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Abstract

Background: Perturbed hematopoietic stem cell (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, reports suggests 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, mRNA expression analyses revealed that miR-199b is markedly enriched in hematopoietic stem cells (HSC) compartment, suggesting that miR-199b may regulate HSCs function. Our flow cytometry analyses of bone marrow cells showed elevated frequencies of primitive HSCs and myeloid progenitors in mice harboring genetic deletion of miR-199b.

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

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

Experimental Approach:

In vitro: In this study, we used a colony-forming unit (CFU) assay as validated in vitro that can detect an increase or decrease in the frequency of hematopoietic stem and progenitor cells (HSPCs) proliferation and/or changes in differentiation potential 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 cells to repopulate blood cells in healthy host mice exposed to sublethal irradiation.

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

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

Methods

Figure 1. Experimental design for in vitro colony-forming unit (CFU) assays. Bone marrow and spleens were harvested from N=3 wild type (WT) and miR-199b KO mice. Cells were plated in MethoCult GM 3534 media for myeloid progenitor differentiation or MethoCult GEMM 3434 media that promotes multi-lineage differentiation. Cells were kept at 37°C with 5% CO₂ and replated at 12 day intervals. Manual counting of cells was done after imaging on STEMvision scanner.

Figure 2. Experimental design for in vivo BMT transplantation study. Bone marrow from N=3 WT and KO mice carrying a CD45.2 marker was harvested and transplanted to irradiated WT mice carrying a CD45.1 marker. At 4, 8 and 12 weeks, blood samples were taken from both mice for flow cytometry to determine donor percentage of mature myeloid and lymphoid cells in peripheral blood.

Results

Figure 3. miR-199b deletion decreases colony forming abilities of splenic HSPCs at first plating. A) Representative images of colony forming assays for splenic HSPCs on GM differentiation medium (CFU-GM) performed in WT and miR-199b KO mice (n=3) for 2.5 and 5.0 x 10⁵ cells. B) Representative images of colony forming assays for granulocyte, monocyte, erythroid, myeloid and megakaryocytes differentiation medium (CFU-GEMM) performed in splenic cells from WT and miR-199b KO mice (n=3) for 2.5 and 5.0 x 10⁵ cells. C) Total CFU-GM and CFU-GEMM for splenic cells in WT and miR-199b KO mice.

Figure 4. miR-199b KO mice show increased proliferation of myeloid-committed progenitors. A) Representative images of serial colony-replating assays at secondary and tertiary plating for myeloid-committed cells (CFU-GM) derived from WT and miR-199b KO progeny cells at first plating. Assays were performed for 2.0 x 10⁴ cells from the first plating. B) Representative images of serial colony-replating assays at secondary and tertiary plating for granulocyte, erythroid, myeloid and megakaryocytes progenitors (CFU-GEMM) derived from WT and miR-199b KO mice (n=3) at first plating. Assays were performed for 2.0 x 10⁴ cells. C) Subsequent CFU plating assays for myeloid progenitors in CFU-GM media for 1.0 x 10⁴ and 2.0 x 10⁴ cells. D) Subsequent CFU plating assays for multilineage progenitors in CFU-GEMM media for 2.0 x 10⁴ cells.

Results

Figure 5. miR-199b KO mice show increased splenic myeloid progenitor proliferation as compared to WT mice. A) Representative images of serial colony-replating assays of secondary plating for myeloid progenitors (CFU-GM) performed in spleen cells from WT and miR-199b KO mice (n=3) for 2.0 x 10⁴ cells. B) Representative images of serial colony-replating assays of second plating for granulocyte, erythroid, myeloid and megakaryocytes progenitors (CFU-GEMM) performed in first plated spleen cells from WT and miR-199b knock-out mice (n=3) for 2.0 x 10⁴ cells. C) Subsequent CFU replating assays for myeloid progenitors in CFU-GM media for 2.0 x 10⁴ cells. D) Subsequent CFU replating assays for multi-lineage progenitors in CFU-GEMM media for 2.0 x 10⁴ cells.

Figure 6. miR-199b KO HSCs have a greater ability to repopulate myeloid cells in the irradiated host mice than WT HSCs. Donor chimerism (%) was measured at 4, 8 and 12 weeks post-transplantation of BM from WT and miR-199b KO mice into sublethally irradiated host mice.

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.

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Modeling Clear Cell Renal Cell Carcinoma: Characterization of the Tumor Extracellular Matrix

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Abstract

Renal cell carcinoma (RCC) is the most common kidney cancer, affecting nearly 64,000 new patients every year^{2,3}. Clear cell renal cell carcinoma (ccRCC) is the most common form of RCC, yet few treatment options exist for patients with this disease². 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 LCMS mass spectrometry and immunofluorescence. Independent data acquisition and analysis using SWATH identified drastic changes in collagen content between tumor and normal cells. It was further determined that a denatured collagen environment increased cell adhesion and Akt signaling using an *in vitro* cell adhesion assay with the 786-O ccRCC cell line. XL313, a truncated RGDKGE collagen epitope, was shown to be secreted by 786-O cells. Immunohistochemical staining of patient tumors showed aberrant expression of XL313 throughout specific ccRCC tumors. These findings suggest that we have designed a cell isolation method that is viable to be used to create a ccRCC model to study cancer-ECM interactions. Additionally, the findings show a potential role of denatured collagen in ccRCC growth.

Introduction

- Developing a ccRCC model requires isolation of ccRCC cells; cells were isolated from tumors, grown in culture, and depleted of fibroblasts.
- Three possibilities exist regarding how ECM changes; new ECM deposited by tumors, ECM is degraded, or ECM is remodeled and exists in an alternate form. We used mass spectrometry and immunohistochemistry to explore these options.
- 786-Os, a ccRCC cell line, were grown on normal and denatured collagen IV to study which environment is more favorable for cancer growth. Secretion of collagen was also studied.
- Remodeling of ECM by damage or proteases can release previously hidden collagen epitopes that affect angiogenesis. In this study we targeted XL313, a collagen epitope linked to increased angiogenesis and inflammation in other cancer types and shown to be secreted by macrophages¹.

Effects of remodeling on cryptic epitopes

Representation of distinct cryptic regulatory elements identified in the ECM protein collagen

Adapted with permission from Dr. Brooks

Methods

- Cell isolation and characterization plan:

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

Results

Figure 1.

Figure 1: A) Summary of specific ccRCC patient diagnostics regarding used for current study. B) Validation staining of ccRCC cell isolation. After culture for three passages, cells were collected using cytopsin and stained for ccRCC markers (CK18 and Vimentin) and fibroblast markers (Vimentin and PDGFR β) and costained with DAPI.

Figure 2.

Figure 2: A) PCA analysis of tumor and normal mass spectrometry data after mass spectrometry acquisition. Noted distinct clustering of tumor and normal cells. B) Differences in core ECM proteins between tumor and normal generated based on p-values <=0.05 from SWATH analysis. Overlapping collagen proteins were identified based on peak abundance analysis of the non-significant pool.

Figure 3.

Figure 3: A) 786-O cells were seeded on natural and denatured collagen IV. Cell adhesion was measured using optical density. B) Western blot of downstream signaling molecules involved in α V integrin signaling pathway following seeded onto natural or denatured collagen IV. C) Western blot of 786-O conditioned media 786-Os after 72 and 48 hours identified XL313 (~15kDa). Raw, a macrophage cell line, was used as an XL313 positive control.

Results

Figure 4.

Figure 4: A) H&E sections of ccRCC. B) Trichrome staining. C) Immunofluorescence of paraffin sections for XL313 collagen epitope. 20x. Insert, 40x. Images were taken of matching regions for each tumor, scale bars 200 μ m and 50 μ m in 40x.

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 α 5 integrin signaling.
- Secretion of denatured collagen XL313 by 786-Os suggests a novel role of XL313 in ECM remodeling in ccRCC.
- XL313 collagen epitopes are highly expressed in tumors Tp18-S108 and Tp18-S109, but not in Tp18-S114, indicating both intratumor and patient heterogeneity.

Future Directions

- Differential adhesion onto denatured collagen repeated with isolated primary ccRCC cells.
- Confirm that XL313 is secreted by primary ccRCC cells, as well as other ccRCC cell lines.
- Staining for α v alongside other TME component cells should be performed to validate ability for cells to interact with XL313 epitope.
- Treatment of cell lines with anti-XL313 and measuring effect using a collagen invasion assay.
- Xenograft 786-O cells into immune compromised mice and treatment with XL313 antibody to understand the role of XL313 on tumor growth *in vivo*.

Acknowledgements

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1. Ames, J. J., Contois, L., Carver, J. M., Tweedie, E., Yang, X., Friesel, R., ... Brooks, P. C. (2016). Identification of an Endogenously Generated Cryptic Collagen Epitope (XL313) That May Selectively Regulate Angiogenesis by an Integrin α 5-associated Protein (VAP) Mechano-transduction Pathway. *The Journal of Biological Chemistry*, 291(10), 2731–2750. <https://doi.org/10.1074/jbc.M115.669614>

2. Hsieh, J. J. et al. Renal cell carcinoma. *Nat. Rev. Dis. Primers*, 3, 17009 (2017).

3. Nahi, S., Kessler, E. R., Bernard, B., Haig, T. W., & Lam, E. T. (2016). Renal cell carcinoma: a review of biology and pathophysiology. *P1000Research*, 7, 307. <https://doi.org/10.12688/P1000Research.13179.1>

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

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Abstract

- The opioid epidemic is a growing public-health concern, particularly in Maine [1], and misuse of opioids can lead to cardiopulmonary arrest [2]. In this study, we evaluated the incidence of opioid-related cardiac arrest (ORCA) admitted to Maine Medical Center (MMC) during 5 years between January 2013 and December 2017, and the differences in baseline characteristics, hospital course, and outcomes between ORCA and non-opioid-related cardiac arrest (non-ORCA) patients.
- A retrospective review was performed on medical records of MMC cardiac arrest patients and in prospectively collected registry data. An arrest was classified as an ORCA if it had no alternative explanation and met at least one of the following criteria: 1) EMS suspected the arrest was opioid-related, 2) the physician team suspected the arrest was opioid-related, or 3) an opioid-positive toxicology screen [3]. Statistical methods used include the Wilcoxon rank-sum test and Fisher's exact test. A P-value less than or equal to 0.05 was considered significant.
- Of 497 patients 51 (10.3%) had ORCA. ORCAs at MMC had increased from 15 in 2013-14 to 26 in 2016-17, a 73% increase. ORCA patients were significantly younger and healthier than other cardiac arrest patients but were more likely to have withdrawal of life support due to severe brain injury. There was no significant difference in functional outcome and in-hospital mortality.
- Admissions to MMC for opioid-related cardiac arrest rose dramatically over 5 years. The demographics and clinical characteristics of opioid-related cardiac arrest differ from other forms of cardiac arrest, while outcomes were similar. More research into the pathophysiology of ORCA is needed to better treat this population.

Introduction

Opioid-related cardiac arrest (ORCA)

Figure 1. Venn diagram representing the unknown similarities and differences between ORCAs and non-ORCAs.

Methods

Figure 2. Method for classifying cardiac arrests as ORCA or non-ORCA. An arrest was considered ORCA if it met at least one of the following criteria with no alternative explanation: 1) EMS suspects ORCA, 2) Physician team suspects ORCA, 3) Opiate-positive toxicology screen. Based on reference [3].

- A retrospective chart review was performed for patients admitted to MMC for cardiac arrest from January 2013 to July 2018.
- Each arrest was classified as ORCA or non-ORCA using a system based on the criteria described in [3].
- Demographic, hospital course, and outcomes data collected from the International Cardiac Arrest Registry (INTCAR).
- These characteristics were compared between ORCA and non-ORCA patients.
- Statistical analyses used include Fisher's exact test and the Wilcoxon signed-rank test.
- A P-value less than or equal to 0.05 was considered significant.

Hypotheses

- The rate of ORCAs increased at MMC and statewide from 2013 to 2018
- ORCA patients demographics and clinical features differ from other cardiac arrests
- ORCA patients have more functional outcomes than non-ORCA cardiac arrest patients
- The pathophysiology of ORCA differs from other cardiac arrest populations

Results

Incidence of ORCA at MMC 2013-2017

Figure 4. Incidence of ORCA at MMC 2013-2017.

Rate of ORCA and non-ORCA between January 2013 and July 2018

Figure 5. Rate of ORCA and non-ORCA between January 2013 and July 2018.

Table 1. Demographics and clinical features of non-ORCA and ORCA patients.

Demographics	Non-ORCA (n = 446)	ORCA (n = 51)	P value
Age (years) [mean(SD)]	59.4 ± 15.8	40.7 ± 14.5	<0.0001
Female	142 (32%)	14 (27%)	0.6
Pre-arrest diseases			
Previously healthy	55 (12%)	13 (25%)	0.016
Arrhythmia	84 (19%)	2 (4%)	0.005
Heart failure	77 (17%)	2 (4%)	0.014
Myocardial infarction	60 (13%)	3 (6%)	0.18
CCI [median (IQR)]	3 (1, 5)	0 (0, 1)	<0.0001
Comorbid conditions	2 (1, 3)	0 (0, 1)	<0.0001
CPC 1 prior to arrest	397 (90%)	49 (98%)	0.07
Clinical features			
Out-of-hospital arrest	327 (74%)	47 (94%)	0.0008
Witnessed	346 (79%)	27 (55%)	0.0006
Initial rhythm VT/VF	220 (52%)	7 (15%)	<0.0001
Bystander CPR	81 (20%)	8 (20%)	0.26
Poor GCS motor subscore	313 (73%)	37 (77%)	0.6
Total ischemic time (min) [median (IQR)]	17 (10.3, 27)	16 (10, 25)	0.9
No-flow interval (min)	4 (1, 7)	5 (1.5, 8.5)	0.16
Shock on admission	285 (64%)	30 (59%)	0.5

Unless otherwise reported, data are presented as number and percentage.
Abbreviations: CCI - Charlson Comorbidity Index; CPC - Cerebral Performance Category; VT/VF - ventricular tachycardia/ventricular fibrillation; CPR - cardiopulmonary resuscitation; GCS - Glasgow Coma Scale

Table 2. Hospital course for non-ORCA and ORCA patients.

Treatments/Tests	Non-ORCA (n = 446)	ORCA (n = 51)	P value
TTM administered	440 (99%)	50 (98%)	0.42
Target temperature (°C)			
33	63 (14%)	4 (8%)	0.28
34	4 (1%)	0	1 (NS)
35	29 (7%)	1 (2%)	0.35
36	24 (5%)	2 (4%)	1 (NS)
Coronary angiography	161 (37%)	5 (10%)	9.99E-05
PCI	74 (17%)	2 (4%)	0.013
Vasopressors / inotropes used during ICU stay	409 (93%)	44 (88%)	0.155
Mechanical circulatory support	424 (97%)	48 (94%)	0.2
Echo (within 12 h ROSC)	104 (23%)	10 (20%)	0.6
Normal >50%			
Moderately impaired 30-49%	90 (20%)	2 (4%)	0.0022
Severely impaired <30%	83 (19%)	5 (10%)	0.173
Echo not done	162 (36%)	33 (65%)	0.000124
MRI	21 (4%)	93 (21%)	0.0022
If MRI, when (days post-ROSC) [median (IQR)]	4 (3, 5)	4 (3, 5)	0.9
Complications			
Pneumonia	153 (34%)	26 (51%)	0.021
Seizures - clinical			
None	362 (82%)	33 (66%)	0.0134
Yes partial	11 (2%)	1 (2%)	1
Yes generalized	50 (11%)	13 (26%)	0.0065
Yes subtle / non-convulsive	5 (1%)	3 (6%)	0.038
Yes before temperature management	19 (4%)	4 (8%)	0.28
Yes during temperature management	40 (9%)	9 (18%)	0.075
Yes after temperature management	10 (2%)	4 (8%)	0.044
Myoclonus	248 (56%)	22 (44%)	0.13

Data are presented as number and percentage.
Abbreviations: TTM - targeted temperature management; PCI - percutaneous coronary intervention; ICU - intensive care unit; ROSC - return of spontaneous circulation; MRI - magnetic resonance imaging

Results

Table 3. Outcomes of non-ORCA and ORCA patients.

Outcome	Non-ORCA (n = 446)	ORCA (n = 51)	P value
Hospital LOS [median (IQR)]	6 (3, 12)	8 (4, 13)	0.048
ICU LOS [median (IQR)]	4 (3, 7)	6 (4, 10)	0.0068
Condition at hospital discharge			
Dead	251 (56%)	28 (55%)	0.155
GCS<8/noninterative	5 (1%)	2 (4%)	0.7
Disoriented	16 (4%)	2 (4%)	0.7
Oriented	174 (39%)	19 (35%)	0.6
Agitated	2 (0.4%)	1 (2%)	0.28
Calm	39 (9%)	3 (6%)	1 (NS)
Nonambulatory	21 (5%)	2 (4%)	0.4
Ambulatory	146 (33%)	14 (27%)	0.9
CPC at hospital discharge			
1	46 (10%)	3 (6%)	0.5
2	94 (21%)	11 (22%)	1 (NS)
3	41 (9%)	5 (10%)	0.8
4	5 (1%)	2 (4%)	0.155
5	255 (57%)	30 (59%)	0.9
CPC = good	140 (32%)	14 (27%)	0.6
Discharge disposition			
Home	93 (21%)	11 (22%)	0.9
Inpatient rehabilitation	66 (15%)	7 (14%)	1 (NS)
Inpatient ventilator unit	2 (0.4%)	0	1 (NS)
Skilled nursing	20 (4%)	2 (4%)	1 (NS)
Hospice	2 (0.4%)	0	1 (NS)
Died after withdrawal of life support	220 (49%)	25 (49%)	1 (NS)
Died despite full support	30 (7%)	5 (10%)	0.39
Life support withdrawn	229 (53%)	26 (55%)	0.9
If yes, cause of WLST			
Severe brain injury	169 (57%)	26 (51%)	0.0075
Patient intent not to be treated aggressively	69 (23%)	3 (6%)	0.11
Multi-organ failure	44 (15%)	19 (37%)	0.000542
Pre-existing advance directives	13 (4%)	1 (3%)	1
Unstable circulation	41 (14%)	19 (37%)	0.000169
Medical co-morbidity	39 (13%)	1 (3%)	0.15
If yes, day of WLST			
Within 48 hours from admission	87 (20%)	5 (10%)	0.126
Day 3-4	60 (13%)	5 (10%)	0.66
Day 5-6	32 (7%)	3 (6%)	1 (NS)
After day 6	51 (11%)	14 (27%)	0.0034

Unless otherwise reported, data are presented as number and percentage.
Abbreviations: LOS - length of stay; ICU - intensive care unit; GCS - Glasgow Coma Scale; CPC - Cerebral Performance Category; WLST - withdrawal of life support

Conclusions

- The incidence of ORCAs at MMC has increased by 73% from 2013 to 2017.
- ORCA patients were younger with fewer comorbid conditions.
- ORCA patients were less likely to have VT/VF.
- ORCA patients were likely to have more severe brain injuries.
- ORCA patients were less likely to undergo additional cardiac interventions.
- There was no difference in ischemic time or Glasgow Coma Scale motor subscore on presentation.
- There was no difference in the rate of in-hospital mortality and good functional outcome between ORCA and non-ORCA patients.

Figure 10. Venn diagram representing the similarities and differences between ORCA and non-ORCA patients.

Figure 7. Additional interventions for non-ORCA and ORCA patients.

Figure 8. Hospital and ICU LOS for non-ORCA and ORCA patients.

Figure 9. Condition at hospital discharge for non-ORCA and ORCA patients.

Next Steps

- Compare patterns of brain injury by serology and MRI between ORCA and non-ORCA patients
- Characterize ORCAs geographically throughout Maine

References

- Russell, E. (2018, February 23). Drug overdoses killed a record 418 people in Maine last year. Retrieved from <https://www.pressherald.com/2018/02/23/drug-overdoses-killed-418-people-in-main-last-year-up-11-percent/>
- Sakuma, A., Szilagyi, M., Vallabhajosyula, S., Cheungpasitporn, W., Patch, R., & Jentzer, J. (2017). National trends and outcomes of cardiac arrest in opioid overdose. *Resuscitation*, 121, 84-88.
- Elmer, J., Lynch, M., J., Kristan, J., Morgan, P., Gerstel, S. J., Callaway, C. W., & Rittenberger, J. C. (2015). Recreational drug overdose-related cardiac arrests: Break on through to the other side. *Resuscitation*, 89, 177-181. doi:10.1016/j.resuscitation.2015.01.029

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

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ABSTRACT

The aim of this project was to investigate the effects of Spry1 deficiency on adipose tissue responses to a Western Diet. Spry1 is highly expressed in the adipocytes and adipocyte progenitors. We looked for a specific "coronal structures" around adipocytes that are indicators of fibrosis. We observed decreased coronal structures in the epididymal adipose tissue of Spry1^{-/-} mice 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 lumen. This activation then induces a phenotypic transition of the smooth muscle cells, from a dormant, contractile state, to a proliferative and migratory, synthetic state.

Our lab has established that Spry1, a regulator of receptor tyrosine kinase (RTK) signaling, plays a critical role in the maintenance of the contractile VSMC phenotype by inhibiting their migration. Spry1 is highly expressed in both the fibroblasts and smooth muscle cells in the vasculature of the heart, as well as in the adipocyte cells.

MATERIALS and METHODS

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/or aging, and based upon our observed increase in F4/80 immunostaining we expected fibrosis to be higher in Spry1^{-/-} mice fed a WD. The unexpected presence of less fibrotic adipose tissue in the Spry1^{-/-} mice (especially eWAT) suggests Spry1 deficiency disrupts the development of fibrosis under hypercholesterolemic conditions. Understanding the role of Spry1 in adipose tissue responses to a WD requires further study and may provide unique opportunities for the treatment obesity, diabetes and cardiovascular disease.
- It appears that Spry1^{-/-} mice, while they have increased atherosclerosis, also have more normal (healthier) looking adipose tissue when fed a Western Diet.

References

- Yang, X., Gong, Y., He, Q., et al., (2017). Loss of Spry1 attenuates vascular smooth muscle proliferation by impairing mitogen-mediated changes in cell cycle regulatory circuits. *Journal of Cellular Biochemistry*, 119(4), 3267-3279. doi:10.1002/jcb.26486
- Wayne Orr, A., Hastings, 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), 168-180. doi:10.1159/000250095
- Shankman, L. S., Gomez, D., Cherepanova, 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), 217-217. doi:10.1038/nm0216-217a

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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
Create a way to assess needs of physician population
2. **Integrated feedback mechanisms:**
How will you determine what they learned?
Review assessment tools available + their validity
3. **Detailed instructional methods:**
How will you teach them this material?

Writing a Curriculum, University at Buffalo Office of Continuing Medical Education, http://medicine.buffalo.edu/cme/planning_cme/writing_a_curriculum.html

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

Figure 1. Three tiers of Emergency Point-of-Care Ultrasound

Core Applications	
Tier 1 (>80% consensus)	<ul style="list-style-type: none"> • FAST • ID of early intrauterine pregnancy • Evaluation of abdominal aorta • Emergent Cardiac Imaging • Procedural guidance of ED Procedures
Tier 2 (>50% consensus)	<ul style="list-style-type: none"> • eFAST • Biliary imaging • Renal imaging • Evaluation of DVT • Thoracic imaging • Soft tissue+musculoskeletal imaging
Tier 3 (<50% consensus)	<ul style="list-style-type: none"> • All other applications

All scan images from: <https://www.acep.org/sonoguide/>

2 surveys and one structured interview were developed to assess the ultrasound training needs of Maine emergency physicians.

Adapted from Henwood et al, *Annals of Emergency Medicine*, 2014

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

2 Simulations

2 Other

2 Multiple-Choice Tests

20 OSCE/SDOT Checklists

Figure 3. The Objective Structured Assessment of Ultrasound Skills (OSAUS)

	1	2	3	4	5
1. Indication for the Examination If applicable. Reviewing patient history and knowing why the examination is indicated	Displays poor knowledge of the indication for the examination		Displays some knowledge of the indication for the examination		Displays ample knowledge for the indication for the examination
2. Applied knowledge of ultrasound equipment Familiarity with the equipment and its functions, i.e. selecting probe, using buttons and application of gel	Unable to operate equipment		Operates the equipment with some experience		Familiar with operating the equipment
3. Image optimization Consistently ensuring optimal image quality by adjusting gain, depth, focus, frequency etc	Fails to optimize images		Competent image optimization but not done consistently		Consistent optimization of images
4. Systematic examination Consistently displaying systematic approach to the examination and presentation of relevant structures according to guidelines	Unsystematic approach		Displays some systematic approach		Consistently displays systematic approach
5. Interpretation of images Recognition of image pattern and interpretation of findings	Unable to interpret any findings		Does not consistently interpret findings correctly		Consistently interprets findings correctly
6. Documentation of examination Image recording and focused verbal/written documentation	Does not document any images		Documents most relevant images		Consistently documents relevant images
7. Medical decision making If applicable. Ability to integrate scan results into the care of the patient and medical decision making	Unable to integrate findings into medical decision making		Able to integrate findings into a clinical context		Consistent integration of findings into medical decision making

Sample SDOT Checklist for Ultrasound Competency Assessment

Tolsgaard et al, *PLoS One*, 2013

Resource Guide for CME in POCUS:

- Summary of expert guidelines for core applications and establishment of 3 tiers according to consensus opinion
- Needs assessment tool to determine training goals
- Compilation of previous assessment tools for each Tier 1 ultrasound application

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

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Time Course of Cognitive Change after Severe Hypoglycemia

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Abstract

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

Introduction

Type 1 Diabetes

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

Methods

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

Adapted from ImPACT Applications, Inc., 2018

Adapted from ImPACT Applications, Inc., 2018

Adapted from ImPACT Applications, Inc., 2018

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.

Figure 1. Symptom scores of participants who had experienced an episode of Grade 4HG; 2013. Used with permission from Irwin Brodsky, MD.

Figure 3. Verbal memory scores of participants who had experienced an episode of Grade 4HG, and control subjects; 2013. Used with permission from Irwin Brodsky, MD.

Figure 2. Scores of visual motor speed, verbal memory, and cognitive efficiency of participants who had experienced an episode of Grade 4HG; 2013. Used with permission from Irwin Brodsky, MD.

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.

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Needs Assessment and Quality Improvement of Pre-Transplant Patient Education

Elisabeth Lualdi, Dr. Paul Han, Jessica Begley, Dr. James Whiting
for the Maine Transplant Center

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.

A needs assessment was conducted to explore ways to improve the educational system at MTC. Findings from a literature review and key informant interviews with MTC clinicians and patients revealed many gaps in MTC patient education.

Using the SDM and TTM models as conceptual frameworks for redesigning the system, an alternative educational pathway to treatment was proposed.

The educational class has been reformatted into an online, modular based program offered to patients as an alternative. At the same time, educational handouts are being refined to enhance readability.

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

- Patient Shadowing
- Class Attendance
- Literature Review
- Patient Phone Interviews: recent class attendees
- Staff Interviews
- Presentation to Transplant Team
- Self-education on health literacy
- CDC scanning of content delivered during class (power point and handouts)

3 Skelton's Recommendations for Educational Programs

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

Results

MTC Clinician Interviews

- Patients should be given access to more resources for information.
- Due to the volume of new referrals, patients have to wait a significant amount of time before they are booked for a class.
- Patients are overwhelmed with information.
- Patients have low health literacy.

Patient Interviews

- Only 45% of patients responded correctly to a fundamental piece of information: that you have to take anti-rejection medications for the life of your transplant. More than 50% of patients reported being very unsatisfied with the progress of their treatment.
- Only 33% had completed some clearances (explained in the class) prior to Visit 1.

Literature Review

- Using TTM, patients educated according to their specific readiness stage are 2 times more likely to engage in a recommended health behavior in the next 6 months than those with generalized recommendations.⁶
- 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.⁴
- 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.⁵

Prototype: A 5 Part Online Power Point with Audio. Alternative Pathway for Education

- Patient Education on Dialysis: Incorporate Modules 1, Introduction to the Maine Transplant Center and 2, Requirements/Eligibility for Transplant
- Patient Referral: special note to patients who have watched Modules 1 and 2
- Program Introduction Phone Call with Patients: options for education

Patients who have completed Modules 1 and 2 have the chance to complete the Module series and forgo a class at the Transplant Center. Patients complete the 3 remaining modules (3. Transplant Options, 4. Risks and Expectations with Transplant, 5. Financing Transplant) and questions within a few day-time frame.

Patients who did not want to complete the initial modules offered on dialysis and do not want to start the online program are booked for a regular class after their referral is processed. The class follows the same content structure as the modules.

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.

Transplantation: Making an Informed Choice

Is transplantation the best treatment option for you?

Goals of this Module:

- Understand your treatment options.
- Understand the pros and cons of transplantation, compared to dialysis.
- Work with your transplant team to make an educated, shared decision based on your personal values.

SSM and TTM Additions in Introductory Module

References + Acknowledgements

- Elwyn, Glyn et al. A Three-Talk Model For Shared Decision Making: Multistage Consultation Process. 2017.
- Swanson, Aaron. Trans Theoretical Model: Coaching and Curing. 2015.
- Skelton SL, et al. Applying best practices to designing patient education for patients with end-stage renal disease pursuing kidney transplant. 2015.
- Lambert, Kelly et al. A Cross-Sectional Comparison of Health Literacy Deficits Among Patients With Chronic Kidney Disease. Journal of Health Communication. 2015.
- Robinson JK et al. Response Across the Health-Literacy Spectrum of Kidney Transplant Recipients to a Sun-Protection Education Program Delivered on Tablet Computers: Randomised Controlled Trial. 2015.
- Waterman, AD et al. Educating Prospective Kidney Transplant Recipients and Living Donors about Living Donation: Practical and Theoretical Recommendations for Increasing Living Donation Rates. 2016.

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

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Abstract

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

Introduction

Table 1. Review of C2 Nerve Root Transection Techniques

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)

Table 1. An overview of literature describing C2 sectioning technique and location for patients who underwent sacrifice with follow up.

Figure 1&2. Demonstrate the complex anatomy relevant to atlantoaxial fusion including the C2 nerve which is sacrificed in this cohort of patients.

Methods

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

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

Results

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

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

	# of Patients	# Nerves	Time Range	Occipital Pain	Narcotics for head pain?	Type of Narcotic
Early f/u Office Visits	35	66	1-7 months (mean=2.7)	0	0	n/a
Delayed Phone Survey	17	31	0.5-4 years (mean=2.2)	4 of 17	1 of 17	liquid morphine

Table 2. Displays incidence of ON reviewed at the two f/u stages.

Table 3. Results from delayed follow up patients

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

Table 3. Breaks down the responses from the 17 patients with delayed f/u using the ACPA and ICHD-3 scales.

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

- C2 nerve root sectioning technique is poorly understood
- C2 nerve root sacrifice has excellent long term outcome
- Transection technique may influence clinical outcome

Acknowledgements

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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." *J Neurosurg Spine* 8(3): 222-229.
- Kang, M. M., et al. (2012). "C2 nerve root sectioning in posterior C1-2 instrumented fusions." *World Neurosurg* 78(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. Molinari (2010). "C1 lateral mass screw placement with intentional sacrifice of the C2 ganglion: functional outcomes and morbidity in elderly patients." *Eur Spine J* 19(8): 1318-1324.

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

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

Abstract

Mesoderm Specific Transcript (*Mest*) is expressed variably among genetically homogeneous mice and highly induced in adipocytes when mice are fed an obesogenic diet. The correlation of elevated *Mest* expression to increased body weight and fat mass gain implicate *Mest* as a potential epigenetic regulator of diet-induced obesity. In order to understand the regulation and function of *Mest*, a conditional transgenic model was created using *Mest* driven by a CAG promoter which can be activated by tissue-specific expression if 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* transgenic mice expressing *Adipoq*-cre positive mice resulted in the induction of *Mest* in adipocytes at a level consistent to mice fed a high fat diet. Previous studies have shown that endogenous *Mest* is co-regulated with several genes including imprinted genes (*Klf14*) and genes associated with the Wnt (*Sfrp5*) and Tgf- β (*Bmp3*) signaling pathways. Using a transgenic model, we were able to confirm that *Mest* expression plays a role in regulating the expression of *Sfrp5* in gonadal adipose tissue and brown adipose tissue, but not in inguinal adipose tissue. *Sic5a7*, a gene shown to be downregulated in gonadal adipose tissue of *Mest* knockout mice was also shown to be up-regulated in mice overexpressing *Mest*. These studies show that this transgenic model will allow us to better understand the biological mechanism by which *Mest* functions as an epigenetic determinant of diet-induced obesity.

Introduction

Epigenetics & Obesity

Epigenetics is defined as the study of how changes in an organism's physical or nutritional environment initiate biological mechanisms that bring about changes in gene expression, but not the underlying DNA sequence. Using an inbred, genetically identical mouse strain, our lab seeks to understand the biological mechanisms and genes that function as epigenetic regulators for variable development of diet-induced obesity. To characterize these epigenetic regulators, inbred mice were fed a high fat diet in a controlled environment to identify genes associated with variable body weight among individuals. *Mest*, an imprinted gene has been shown to be strongly correlated with increased body weight and fat mass gain.

Highly variable induced expression of *Mest* among genetically homogeneous mice when fed an obesogenic diet presents a strong correlation with an increase in body weight and in fat mass. The highest and lowest 10th percentile of *Mest* expression was quantified by qRT-PCR in arbitrary units (AU) and normalized to cyclophilin.

Adipose-specific inactivation of *Mest*

Mice with an adipose tissue specific inactivation of *Mest* created by crossing mice with a paternal floxed allele for *Mest* with *Adipoq*-cre transgenic mice reduced bodyweight gain and adiposity compared to littermate controls after being fed a high fat diet from 8 to 24 weeks of age.

Methods

Transfection of HEK293 cells CAG-GFP-Mest and CAG-GFP-MestMyc (A,B) and co-transfection of cells with CAG-cre recombinase (C,D) resulted in reduced GFP signal for CAG-GFP vectors. Cells were quantified by qRT-PCR.

Results

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

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

Gene expression was measured in arbitrary units (AU) and normalized by TBP. We determined that mice with transgenic expression of *Mest* also showed elevated levels of *Sfrp5* and *Sic5a7* in gonadal fat and in brown fat only and lower levels of *Ucp1* that was most evident in gonadal fat.

Summary/Conclusions

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

- Analysis by qRT-PCR revealed that *Mest* overexpression in the transgenic model was specific to adipocytes and that it was at a level consistent with HFD-induced wild type *Mest* expression in inguinal and gonadal fat.
- Expression of *Sfrp5* and *Sic5a7* was elevated in gonadal fat and brown fat, but not in inguinal fat. This increase was significant in *Sfrp5* and *Sic5a7* ($p < 0.005$) in brown fat, and in *Sic5a7* ($p < 0.05$) in gonadal fat.
- Mest* transgenic mice also tend to have reduced expression of *Ucp1* in gonadal and brown adipose tissue.

These results suggest that *Mest*, or the consequence of elevated expression of *Mest*, plays some role in the function and regulation of several adipose genes such as *Sfrp5*, *Sic5a7* and *Ucp1*. Further studies using a larger pool of age-matched mice and various dietary conditions, as well as mice with inactivation of *Mest*, will be valuable in determining how transgenic overexpression of *Mest* effects adipose tissue function.

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Podocalyxin Promotes Activation of STAT3 Signaling Pathway in Emergency Granulopoiesis

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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 (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.

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

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

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

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

Methods

We used Podxl conditional knock out (cKO) mice model generated using the flox-Cre technique to delete the expression of Podxl in hematopoietic lineages. Wildtype and Podxl cKO mice were injected with 125 µg/kg G-CSF twice in two days to induce emergency response in hematopoietic system. Initially, induction 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 cKO(cKO), Podxl cKO with G-CSF(cKO G-CSF). Bone marrow(BM) from the tibias and femurs was collected. BM cells were flushed with IMDM medium supplemented with 2% FBS and antibiotic and centrifuged. BM cells were passed through 21G syringe and 0.45 micron strainer. 0.5% Bovine Serum Albumin buffer and PBS 1x was added to the cells to be centrifuged. Cells were counted using hemocytometer. Red blood cells were removed by chemical lysis. Single cell suspension of BM cells was processed by EasySep Mouse Hematopoietic Progenitor Cell Isolation Kit according to manufacturer's protocol (StemCell Technologies). Lineage negative population was obtained using immuno-magnetic separation. Number of lineage negative cells was evaluated. One million cells were blocked with rat and rabbit IgG and labelled by antibodies (C-kit, Sca1, CD16/32, CD34) followed by fixation and permeabilization. Phospho-STAT3, STAT3, and isotype IgG antibodies were added separately to tested groups and incubated for one hour. Samples were submitted to flow cytometry for analysis. Flow cytometry data analysis was performed using FlowJo.

Figure 1: Flowchart diagram portraying experimental design

Results

Figure 2: G-CSF induces GMP proliferation and STAT3 activation in vivo. Lineage negative c-kit positive, Sca-1 negative compartment (KL) containing myeloid progenitors from wildtype mice injected with either G-CSF or PBS, N=4. To characterize GMP population, CD34 and CD16/32 antibodies were used. GMP compartments were further analyzed to show expression of phosphorylated STAT3 [A] or un-phosphorylated STAT3 [B] expression between groups. Anti-STAT3-PE, phospho-STAT3 PE and isotype control-PE antibodies were also used. Plots were collected using FlowJo. Statistical significance was performed using the Student's Two-Tailed T-Test for Significance. *p<0.05 **p<0.01 ***p<0.001.

Figure 3: Loss of Podxl reduces frequency of GMPs during homeostasis and G-CSF-induced stress granulopoiesis. A) Representative gating strategy of lineage negative c-kit positive Sca-1 negative compartment containing myeloid progenitors and subsequent characterization of GMP population between Podxl cKO and WT mice treated with either PBS or G-CSF. In bone marrow, lineage negative population, c-kit, and sca-1 were used to identify myeloid progenitor compartment, and CD34 and CD16/32 antibodies were used to identify GMP populations. Experiments were performed three times. (N=4, N=8, N=8). Both plots were collected using FlowJo. B) Frequency of GMP population between Podxl cKO and wildtype mice treated with either PBS or G-CSF. Statistical significance was performed using the Student's Two-Tailed T-Test for Significance. *p<0.05 **p<0.01 ***p<0.001

Figure 4: Loss of Podxl reduces activity of STAT3 signaling during homeostasis and G-CSF-induced 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<0.01 ***p<0.001. (N=4, N=8, N=8).

Discussion and Conclusions

We found that absence of Podxl in GMPs reduces activity of STAT3 on basal level and in G-CSF-induced response. This negative effect of Podxl deletion on STAT3 phosphorylation coincided with markedly reduced number of GMPs. We found no difference in the total level of STAT3 in G-CSF-treated Podxl cKO as compared to wildtype mice, however total levels of STAT3 were increased in Podxl cKO in steady-state, suggesting an existence of regulatory feedback. Studies have shown that in steady state, STAT3 serves a negative regulatory function by suppressing accumulation of neutrophils in blood¹. This activity is attributed to STAT3-dependent regulation of a feedback inhibition of G-CSFR signaling. We recently found that Podxl deletion increases accumulation of the immature myeloid subpopulation with the associated drop in mature neutrophils in the bone marrow, and increased levels of neutrophils in blood. We found that Podxl-deficient GMPs showed suppressed G-CSF-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 progenitor's cell-cycle progression and maturation in response to G-CSF. We also hope to investigate if Podxl regulates neutrophil trafficking during emergency granulopoiesis.

Figure 5: Model of Podxl's role in G-CSF induced Emergency Granulopoiesis. Upon G-CSF (left panel), Podxl is hypothesized to promote Jak2/STAT3 pathway. Jak2/STAT3 pathway consists of intracellular signaling cascades that control demand-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. Podxl's absence 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 accumulation of neutrophils in blood. The reason for the increase in neutrophil levels in the absence of Podxl during G-CSF-induced response remains to be elucidated.

References

- Lee CK, Raz R, Gimeno R, et al. STAT3 is a negative regulator of granulopoiesis but is not required for G-CSF-dependent differentiation. *Immunity* 2002;17(1):63-72

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

Jonathan Emery, Lee Lucas PhD, Leslie Bradford MD

Aim

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

Introduction

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

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

Methods

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

Exclusion Criteria

Study Variables

PREDICTIVE VARIABLE:

Travel Distance

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

Covariates

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

OUTCOME VARIABLE:

30 Day Mortality

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

Baseline Characteristics of Cohort

Characteristic	1st quartile (n = 41,448)	4th quartile (n = 41,380)	Chi-square p-value
Age			<.0001
< 40	2509 (6)	2565 (6)	
40 - 49	4887 (12)	5177 (13)	
50 - 59	9054 (22)	10420 (25)	
60 - 69	9570 (23)	11601 (28)	
70 - 79	8551 (21)	8294 (20)	
> 80	6875 (17)	3333 (8)	
Charlson-Devy score			<.0001
0	32243 (78)	33496 (81)	
1	7046 (17)	6206 (15)	
> 2	2155 (5)	1626 (4)	
Insurance			<.0001
Uninsured	1706 (4)	1756 (4)	
Private	17486 (42)	18507 (44)	
Medicaid	3093 (7)	2571 (6)	
Medicare	18271 (44)	16705 (40)	
Other	259 (1)	545 (1)	
Median income in patient's ZIP code			<.0001
< 38,000\$	8115 (20)	10179 (25)	
38,000\$ - 47,999\$	9017 (22)	14017 (34)	
48,000\$ - 62,999\$	10289 (25)	10771 (26)	
> 63,000\$	13997 (34)	6345 (15)	
No high school degree in patient's ZIP code			<.0001
> 21%	7497 (18)	8338 (20)	
13% - 20.9%	9122 (23)	13037 (32)	
7% - 12.9%	13026 (31)	13729 (33)	
< 7%	11186 (27)	6187 (15)	
Facility Type			<.0001
Community Cancer Program	3906 (10)	715 (2)	
Comprehensive Community Cancer Program	17852 (46)	13412 (35)	
Academic/Research Program	12849 (33)	21203 (55)	
Integrated Network Cancer Program	4330 (11)	3456 (9)	
Rurality			<.0001
Metro	39334 (97)	21703 (54)	
Small Urban	1043 (3)	6349 (16)	
Rural	212 (1)	9915 (25)	
Isolated Rural	5 (0)	2387 (6)	
Stage			<.0001
1	9620 (24)	9647 (23)	
2	3715 (9)	3877 (9)	
3	15902 (38)	19395 (47)	
4	11809 (29)	8471 (20)	
Mean facility volume per year			<.0001
< 11.6	16634 (40)	4800 (11)	
11.6 - 25.08	11180 (27)	8945 (22)	
25.08 - 39.67	8414 (20)	10515 (25)	
> 39.67	5218 (13)	17330 (42)	

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.

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

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Background

Powassan virus (Flaviviridae: *Flavivirus*) is a vector-borne disease that circulates in parts of North America and Russia in two serologically-indistinguishable lineages: lineage I (POWV) and lineage II (deer tick virus [DTV]). This study looked at DTV. Recent evidence suggests DTV may exhibit nidality, meaning it exists in small microhabitats (foci) consisting of just the right mixture of environmental factors for the virus to propagate.²

- Exists primarily in a **zoönotic** cycle between *Ixodes scapularis* ticks (deer ticks) and small-medium mammals (e.g. the white-footed mouse, woodchucks, and skunks).⁷
- ~10% of POWV human infections result in deadly encephalitis and 50% result in long-term neurological sequelae.⁷
- 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.^{5,7}
- 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 the fall of 2017.

Fig 1: Left image is a map of human POWV/DTV infections in Maine and the right image is showing prevalence of POWV-infected deer ticks

Objectives

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

Hypotheses

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

Methods

Field Collection

- Flagged for ticks in ten transects (5 different habitat types) in the Wells National Estuarine Research Reserve in Wells, ME. Each transect made up of ten consecutive 10x1m plots.

 - Forest with invasive shrub species in the understory
 - Forest with sparse, native shrub species in the Understory
 - Edge (forest-field intersection)
 - Shrub (little-to-no tree cover)
 - Field (grassland)

Fig. 2: Left image is an example of the flagging procedure for tick collection. The right image shows female (F) and male (M) adult deer ticks

Laboratory Testing

- Ticks were ground in PBS solution and aliquots of these were tested in pools (10 adults/pool, 20 nymphs/pool, and 50 larvae/pool) by RT-PCR, amplifying the NS-5 gene (806bp).
- Individual ticks from positive pools were then tested using the same RT-PCR protocol and any positives were confirmed by genomic sequencing
- To determine rate of co-infection, DTV-positive ticks were then tested for *B. burgdorferi*, *A. phagocytophylum*, *B. microti*, and *B. miyamotoi* by PCR assay.

Results

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

Table 1. Field Site Characteristics and Collection Results			
Transect Number	Habitat Type	Number of Ticks (%)	Number of DTV Positives
Transect 1	Brush	23 (6.2)	1
Transect 2	Brush	2 (0.5)	0
Transect 3	Forest with Invasive Understory	106 (28.4)	6
Transect 4	Forest with Invasive Understory	113 (30.3)	2
Transect 5	Field	6 (1.6)	0
Transect 6	Field	10 (2.7)	1
Transect 7	Forest/Edge	14 (3.8)	0
Transect 8	Forest/Edge	21 (5.6)	0
Transect 9	Forest with Native/ Sparse Understory	67 (18.0)	0
Transect 10	Forest with Native/ Sparse Understory	11 (2.9)	0
Total		373	10

Results

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

Fig. 3: Bar graph showing the number of ticks/hr by habitat type. The most adults and nymphs were collected from the forested habitats with invasive shrub species in the understory

An ArcGIS hotspot analysis tool identified, with 99% confidence, that areas within transect 3 (forest with invasive shrub species in the understory) may have been "hotspots" (focal transmission sites) for DTV.⁴

POW hot spots, 99% CI, WNERR, 2018

Fig. 3: Hotspot analysis of transects. Transects are numbered and dots represent plots within transects. The red dots from transect 3 indicate hotspots identified with 99% confidence and white dots indicate plots that were not identified as hotspots

Co-infection PCR assays revealed that seven out of the ten total DTV-positive ticks (70%) were also infected with *B. burgdorferi* (Lyme). None of the DTV-positives were also infected with *A. phagocytophylum*, *B. microti*, or *B. miyamotoi*.

Discussion

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

- This novel finding indicates possible nidality (focality) for DTV that corresponds with habitats known to be favorable for deer ticks (i.e. deciduous forest with invasive shrub species in the understory). Therefore, invasive shrubs, like barberry, may play a role in forming DTV-favorable pathobiocenoses. However, differences in sample sizes between transects means we need to exercise caution when extrapolating this data.
- The habitat type in which the most ticks were collected, deciduous forest with invasive shrub understory, is consistent with previous research.⁸ This is thought to be because some invasive shrub species are resistant to deer browsing, act as good habitats for small mammals, and provide shade for ticks⁶.
- Our reported 2.7% infection rate is within the 0.6-5% range expected from previous reports.³ The actual infection rate may be higher because this study primarily took place during peak nymphal season, and nymphs may have lower infection rates than adults (unpublished data).
- Also similar to previous reports, only lineage II (DTV) and not lineage I (POWV) was found in *I. scapularis* ticks.^{2,3,7}
- The 70% positivity rate for DTV and *B. burgdorferi* (Lyme) co-infected ticks corresponded with reports on Lyme infection rates in Wells, ME (annual CDC report 2012).

References

- Aliota, M. T., A. P. Dupuis, M. P. Wilczek, R. J. Peters, R. S. Ostfeld, and L. D. Kramer. 2014. The Prevalence of Zoonotic Tick-Borne Pathogens in *Ixodes Scapularis* Collected in the Hudson Valley, New York State. *Vector Borne and Zoonotic Diseases* 14: 245-250.
- Anderson, J. F., and P. M. Armstrong. 2012. Prevalence and Genetic Characterization of Powassan Virus Strains Infecting *Ixodes scapularis* in Connecticut. *The American Journal of Tropical Medicine and Hygiene* 87: 754-759.
- Campbell, O. 2018. A Review of Powassan Virus Epidemiology in Ticks and Humans. *Yale University*.
- Environmental Systems Research Institute (ESRI). (2017). *ArcGIS Release 10.5.1*. Redlands, CA.
- Eisen, R. J., L. Eisen, and C. B. Beard. 2016. County-Scale Distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the Continental United States. *Journal of Medical Entomology* 53: 349-386.
- Elias, S. P., C. B. Lubelczyk, P. W. Rand, E. H. Lacombe, M. S. Holman, and R. P. Smith. 2006. Deer browse resistant exotic-invasive understory: an indicator of elevated human risk of exposure to *Ixodes scapularis* (Acari: Ixodidae) in southern coastal Maine woodlands. *Journal of medical entomology* 43: 1142-1152.
- Hernandez, M. E., and S. Thangamani. 2017. Powassan Virus: An Emerging Arbovirus of Public Health Concern in North America. *Vector-Borne and Zoonotic Diseases* 17: 453-462.
- Lubelczyk, C. B., S. P. Elias, P. W. Rand, M. S. Holman, E. H. Lacombe, and R. P. Smith Jr. 2004. Habitat associations of *Ixodes scapularis* (Acari: Ixodidae) in Maine. *Environmental Entomology* 33: 900-906.

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Genetic Testing in Hospitalized Children with ASD: Prevalence and Influences

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for the Autism and Developmental Disorders Inpatient Research Collaborative (ADDIRC)

Introduction

- The American College of Medical Genetics, American Association of Pediatrics, American Association of Psychiatry, and other medical governing bodies recommend that all children with ASD are referred for genetic testing.¹
- Unfortunately, these clinical recommendations are not reflected in practice throughout the ASD population.^{1,2}
- Children with ASD seeking care in inpatient psychiatric units could benefit greatly from genetic testing due to the complex nature of their diagnoses and behavior, which often include lower verbal levels, intellectual disability, self-injurious behavior and emotional dysregulation.³

Objectives

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

Methods

- 936 participants with ASD confirmed by direct assessment using the Autism Diagnostic Observation Schedule (ADOS) were recruited from the Autism Inpatient Collection³, a study conducted in 6 child psychiatric units in the US specializing in treatment of ASD.
- 207 were excluded due to missing information or inconclusive data (N = 729).
- Parents filled out a Demographic and Medical Intake Form at admission which included questions regarding previous treatment, family history and other medical history.
- Parents also filled out an Aberrant Behavior Checklist - Community form at admission, scoring their child on 5 subcategories of behaviors from 0 = never a problem to 3 = severe problem.
- Bivariate logistic regressions were conducted to examine the effect of parent reported genetic testing in hospitalized children with ASD.

Results

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

Demographic	Genetic Testing (N=314)	No Genetic Testing (N=415)	Statistics
Age (Years) (M/SD)	13.32 (3.45)	12.6 (3.34)	P = 0.204
Sex (Male) (N%)	238 (75.7%)	350 (84.3%)	X ² = 8.36 p = 0.004
ADOS Module (N%)			X ² = 21.63 p = 0.000
1	143 (45.5%)	135 (32.5%)	
2	44 (14%)	40 (9.6%)	
3	96 (30.5%)	181 (43.6%)	
4	31 (9.87%)	59 (14.21%)	
Parental Education (N%)			X ² = 1.22 p = 0.269
HS or Less	65 (21.4%)	103 (24.9%)	
At Least Some College	238 (78.5%)	309 (75.1%)	
Adopted (N%)	38 (12.1%)	36 (8.67%)	X ² = 2.30 p = 0.129
Household Income			X ² = 17.44 p = 0.000
Less than \$80,000	186 (62.6%)	282 (72.3%)	
\$80,000 - \$160,000	77 (25.9%)	94 (24.1%)	
More than \$160,000	34 (11.4%)	34 (8.3%)	

- Of the total child sample, 588 (80.6%) were male and the average age was 12.9 (SD = 3.3).
- 436 (54.3%) children were non- or minimally-verbal (determined by ADOS Module administered).
- There were statistically significant differences in gender, ADOS module, and household income between the two genetic testing groups.
- Those with genetic testing were more likely to be non- or minimally-verbal, and have a household income of more than \$160,000.

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

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

Results

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 etiology⁴ and 8.06% were considered ASD candidate genes⁵.

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

- 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% times less likely.

- 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.

Results

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

Conclusions

- Children seeking care in an inpatient psychiatric facility received genetic testing at a higher rate than the general ASD community (<40%),^{1,2} although the diagnostic yield for these tests matched the national standards (10-40%).^{1,5}
- 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

- Barton, Krysta S. et al. "Pathways from Autism Spectrum Disorder (ASD) Diagnosis to Genetic Testing." *Genetics in Medicine: Official Journal of the American College of Medical Genetics* (2017): 10.1038/gim.2017.168. PMC.
- Kiely, Bridget, Sujit Vetter, and Andrew Adelman. "Utilization of Genetic Testing among Children with Developmental Disabilities in the United States." *The Application of Clinical Genetics* 9 (2016): 83-100. PMC.
- Siegel, Matthew et al. "The Autism Inpatient Collection: Methods and Preliminary Sample Description." *Molecular Autism* 6 (2015): 61. PMC. Web. 25 July 2018.
- Scheffer, G. Bradley and Nancy J. Mendelson. "Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions." *Genetics in Medicine: 15* (2013): 399-407.
- "Gene List- April 2017." *Spark for Autism*. www.sparkforautism.org

Acknowledgements

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

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Center for Outcomes Research and Evaluation (CORE) at Maine Medical Center Research Institute (MMCRI)

Abstract	Methods	Key Content Elements of Discussions	Conclusions																						
<p>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.</p> <p>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.</p>	<ul style="list-style-type: none">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? Identify three (3) essential things the patient needs to know."Frequencies of multiple-choice responses were tabulated.Analysis of open-ended items was conducted using qualitative methods.<ul style="list-style-type: none">Software-assisted coding with MAXQDA™	<p>"Given what you know about GTT, how would you introduce it to a patient?"</p> <div><div>Key Elements of Physician-Patient Discussions</div><div><div>Nature of GTT (n=201)</div><div>Uncertainty about GTT (n=59)</div><div>Patient Expectations (n=24)</div></div><div><div>GTT Background Information (n=48)</div><div>Potential Outcomes of GTT (n=25)</div><div>Uncertainty about Therapeutic Options (n=26)</div><div>Uncertainty from Incomplete Evidence (n=10)</div></div><div><div>Logistics of Testing (n=43)</div><div>Therapeutic Implications (n=43)</div><div>Genetics & Genomics (n=21)</div><div>Discovery of Germline Variants (n=11)</div><div>Potential Harms (n=7)</div></div></div>	<ul style="list-style-type: none">Cancer care providers identify several different goals for physician-patient discussions about GTT<ul style="list-style-type: none">The most commonly prioritized goals relate to informed and shared decision making, and managing patient expectations.Providers identify a variety of different content elements for physician-patient discussions about GTT: nature of GTT, uncertainties about its value for treatment decisions, and patient expectations<ul style="list-style-type: none">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:<ul style="list-style-type: none">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.																						
Background	Results	Illustrative Quotations from Open-ended Responses																							
<ul style="list-style-type: none">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 state-wide research project aimed at disseminating and implementing GTT in community oncology practices throughout the state of Maine.	<table><thead><tr><th>Participants</th><th>n</th></tr></thead><tbody><tr><td>Oncology physicians</td><td>17</td></tr><tr><td>Registered nurses</td><td>14</td></tr><tr><td>Genetic counselors</td><td>5</td></tr><tr><td>Pathologists</td><td>5</td></tr><tr><td>Other (e.g. practice administrators, genetic counselors, other physician specialists)</td><td>35</td></tr><tr><td>Total</td><td>76</td></tr></tbody></table>	Participants	n	Oncology physicians	17	Registered nurses	14	Genetic counselors	5	Pathologists	5	Other (e.g. practice administrators, genetic counselors, other physician specialists)	35	Total	76	<table><thead><tr><th></th><th></th></tr></thead><tbody><tr><td>Discuss nature of GTT</td><td><ul style="list-style-type: none">"Every cancer is unique / This is a way to utilize precision medicine and offer a more personalized treatment based on cancer genomics and identifying specific genes and mutations associated with patient's individual cancer""Testing the cancer genome and not germline/ Looking for an unusual and novel alteration in the genome that may give us insight into additional ways to treat your cancer"</td></tr><tr><td>Convey uncertainty about GTT</td><td><ul style="list-style-type: none">"The chance of finding a practical – treatment is small but if found could lead to major benefit""Emerging technology; some meaning well understood, some not""There may be no information that helps at this time but potential for future treatment"</td></tr><tr><td>Maintain expectations about GTT</td><td><ul style="list-style-type: none">"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"</td></tr></tbody></table>				Discuss nature of GTT	<ul style="list-style-type: none">"Every cancer is unique / This is a way to utilize precision medicine and offer a more personalized treatment based on cancer genomics and identifying specific genes and mutations associated with patient's individual cancer""Testing the cancer genome and not germline/ Looking for an unusual and novel alteration in the genome that may give us insight into additional ways to treat your cancer"	Convey uncertainty about GTT	<ul style="list-style-type: none">"The chance of finding a practical – treatment is small but if found could lead to major benefit""Emerging technology; some meaning well understood, some not""There may be no information that helps at this time but potential for future treatment"	Maintain expectations about GTT	<ul style="list-style-type: none">"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"
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Flow Cytometric Analysis of Circulating Microparticles After Cardiac Arrest

centered around you

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INTRODUCTION

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

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

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

METHODS

Study participants: Research was performed in accordance with study protocols approved by Maine Medical Center Institutional Review Board, which is accredited by the Association for the Accreditation of Human Research Protection Programs (AAHRPP). Post-CA subjects age 18 years or older, admitted to the ICU after a cardiopulmonary arrest and treated with Targeted Temperature Management were enrolled after informed consent of the medicolegal Power of Attorney. Subjects underwent phlebotomy at 6, 12, 24, 48, 72 and 168 hours after Return of Spontaneous Circulation (ROSC). Control subjects underwent Coronary Artery Bypass Graft (CABG) surgery. Inclusion criteria included patients 18 years of age or older scheduled for open heart surgery supported by cardiopulmonary bypass (CPB) at Maine Medical Center.

Plasma Samples: Venous blood (10 ml) was collected from cardiac arrest and control CABG subjects using BD Vacutainer ACD tubes. Platelet-free plasma was prepared at room temperature using two-step centrifugation, each at 2,000g for 20 minutes. After preparation plasma was stored at -80°C until further analysis.

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

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

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

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

RESULTS

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

Figure 2. The number of circulating microparticles in CA subjects at 6 hours is significantly higher compared 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.

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.

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.

Sympathetic and sensory innervation of bone modulates remodeling

Nick Banks; Audrie Langlais BS; Audrey Bergeron MS; Roni Kunst MS ; Adriana Lelis 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 years². Studies of β -adrenergic ligands and bone density have well established an inverse relationship between sympathetic nervous output and bone metabolism (Figure 1)⁵. 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 disease⁴. We use immunohistochemical staining techniques to elucidate the phenotypes, distribution and organization of nerve fibers in bone. Semiconductor nanocrystals (quantum dots) have more desirable optical qualities than organic dyes and fluorophores⁶, 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 this equilibrium towards increased resorption and decreased bone formation. The myriad of factors by which the nervous system interacts with the skeleton (hormones, peptides, neurotransmitters [serotonin], etc.) comprise a somewhat "black box" type system. There is observable input and output, but incomplete understanding of the internal workings. The use of immunofluorescent neural markers seems promising in shedding light on this system. However, immunohistochemistry (IHC) and confocal imaging of bone present difficulties. The requisite fixation time and decalcification reduces antigenicity. This diminishes signal strength, which in addition to the high auto fluorescence and low neuron density of bone has lead to few labs reliably able to perform these techniques routinely¹. An efficient, reproducible technique for visualizing and quantifying the differential innervation of bones will be quintessential in understanding the complex network between bone and brain³.

Figure 1. Sympathetic control of bone response⁴.

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

Methods and Materials

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

Figure 3. Indirect Immunofluorescence with Quantum Dot

Figure 4.

Figure 5. Flow chart of standard skeletal preparation with changes in orange

Results

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

Figure 7. Inconclusive staining for sympathetic innervation in growth plate of Tibia/Fibula. Tyrosine hydroxylase (TH) is an enzyme involved in the production of catecholamines and localizes sympathetic nerve fibers. Maximum intensity projection of confocal 10 µm z-stack, 20x objective.

Discussion

Unfortunately, we have not yet optimized technique enough to isolate meaningful signal from background, although positive staining has generally been in the reasonable location but lacks any "nerve like" morphological characteristics. Must titrate to detergent concentration, antibody type/dilution/incubation times to optimize staining. Rigorous negative controls and positive controls (with 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 9) of multiple neural markers and receptors (TH, CGRP, TRPM8,) to show colocalization, giving clues to communication mechanisms.
- Quantitative comparison of neural organization and density between long bones and vertebrae.
- Study changes in neural density from neuropathy in mouse BTBR Ob/Ob diabetes model.

Figure 8. 3d Reconstruction (Autoquant) of Figure 7 with potential neurons traced with Imaris "filament" algorithm

Figure 9. Design for single UV excitation multiplex assay for variety of neurochemical phenotypes

B3 Tubulin
(Pan neuronal marker)
TRPM8
(Cold receptor)
CGRP
(+Peptide Sensory Neurons)
Tyrosine Hydroxylase
(Sympathetic Neurons)
TrkA
(Sensory Neurons)

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References

- Chartier, S., Thompson, M., Longo, G., Fealk, M., Majuta, L. and Mantyh, P. (2014). Exuberant sprouting of sensory and sympathetic nerve fibers in nonhealed bone fractures and the generation and maintenance of chronic skeletal pain. *Pain*, 155(11), pp.2323-2336.
- Dimitri, P. and Rosen, C. (2016). The Central Nervous System and Bone Metabolism: An Evolving Story. *Calcified Tissue International*, 100(5), pp.476-485.
- Kusumbe, A., Ramasamy, S., Stasichova, A. and Adams, R. (2015). Sample preparation for high-resolution 3D confocal imaging of mouse skeletal tissue. *Nature Protocols*, 10(12), pp.1904-1914.
- Motyl, K. and Barbe, M. (2018). *Bone and the Brain* (Chapter 18).
- Motyl, K. and Rosen, C. (2012). The Skeleton and the Sympathetic Nervous System: It's about Time! *The Journal of Clinical Endocrinology & Metabolism*, 97(11), pp.3908-3911.
- Rosenthal, S., Chang, J., Kovtun, O., McBride, J. and Tomlinson, I. (2011). Biocompatible Quantum Dots for Biological Applications. *Chemistry & Biology*, 18(1), pp.10-24.

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

Fig. 1. Differential phenotype of perivascular adipose tissue in healthy individuals vs. cardiovascular disease.

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 vascularization 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

Fig. 2. Proteomic assay performed on tissues collected from *M. musculus* fed a HFD or CD for 12 weeks.

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

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

Methodology

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

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

Gene	Name	gWAT CD	gWAT HFD	BAT CD	BAT HFD	PVAT CD	PVAT HFD
S27A2	Very long-chain acyl-CoA synthetase	1,041	6,294	19,618	33,436	2,436	16,485
HMCS2	Hydroxymethylglutaryl-CoA synthase	6,771	50,776	2,399	6,749	2,115	11,594
PLIN2	Perilipin-2	16,034	219,927	19,137	33,800	14,437	41,056
ANXA1	Annexin A1	1,311,051	2,012,223	84,678	138,520	49,419	132,267
HSDL2	Hydroxysteroid dehydrogenase-like protein 2	142,657	156,959	317,538	475,215	136,610	309,144
TAGL2	Transgelin-2	773,866	1,083,330	198,420	293,259	123,726	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^{-ΔΔCt}. Ppia (Cyclophilin A) was used as the reference gene. qRT-PCR reactions contained 10ng of cDNA and 300nM primer concentration. Cycle: 95°C, 3 min; 95°C 15 seconds, 60°C 30 seconds x 40 cycles. n=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, will be further investigated as potential PVAT markers.

Acknowledgements

I'd like to thank Dr. Lucy Liaw and the members of her lab; Larisa Ryzhova, Josh Boucher, Jessica Davis-Knowlton, Anne Harrington, Terry Henderson, Emily Cooper, & Jacqueline Turner for their assistance and support during this project. David Champlin (USM Biology Department Chair) for securing and facilitating funding for the USM fellowship. Cal Vary (MMCRI Faculty Scientist III) for the proteomic analysis and Michele Karolak (MMCRI Molecular Phenotyping Core Manager) for maintaining the 384 Thermocycler with which I have become intimately familiar.

Pediatric Interfacility Transfers – Association of Pre-transfer Vital Signs with Length of Stay at a Tertiary Care Center

Sarah Bunting BA, Leah Mallory MD, Logan Murray MD

Maine Medical Center, Department of Pediatrics, Portland Maine

Background

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

Objectives

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

Materials and Methods

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

References

- Rosenthal JL, Hilton JF, Teufel RJ, 2nd, Romano PS, Kaiser SV, Okumura MJ. Profiling Interfacility Transfers for Hospitalized Pediatric Patients. *Hosp Pediatr*. 2016;6(6):345-353.
- Gattu RK, De Fee AS, Lichenstein R, Teshome G. Consideration of Cost of Care in Pediatric Emergency Transfer-An Opportunity for Improvement. *Pediatr Emerg Care*. 2017;33(5):334-338.
- Simon TD, Cawthon ML, Stanford S, et al. Pediatric medical complexity algorithm: a new method to stratify children by medical complexity. *Pediatrics*. 2014;133(6):e1647-1654.
- Pediatric advanced life support: Provider Manual. Dallas, TX: American Heart Association, American Academy of Pediatrics; 2016.

Transfer Patient Characteristics

Variable	Median [range], or n (%)
n	236
Age (months)	67.5 [0-225]
Male sex	122 (51.7)
Medical complexity:	
Non-chronic	118 (50.0)
Non-complex chronic	80 (33.9)
Complex chronic	38 (16.1)
Admission type:	
Direct to IPUs	78 (33.1)
Direct to PICU	51 (21.6)
Not direct	107 (45.3)
Hospitalist Service Involved	185 (78.4)
LOS (hours)	42.5 [2.6-1392]
Early Discharge/Unexpected Transfer:	
D/C from MMC ED	13 (5.5)
Transfer from IPUs to PICU in 24h	10 (4.2)
Transfer to other hospital in 24h	1 (0.4)

Pre-transfer Vital Signs and Median LOS

¹ Mann-Whitney U test

Results

- Many patients were discharged quickly after transfer; 5.9% in <6 hours; 11.9% in <12 hours; and 30.9% in <24 hours.
- Most vital signs were reliably obtained (HR for 93.6% of patients; RR, 90.7%; temperature, 87.3%; spO₂, 92.4%). BP was an exception at 47.9%.
- Patients with abnormal RRs before transfer have significantly longer LOS than patients with normal RRs (61 v. 38 hours, $p = 0.017$). The same finding was apparent for BP (57 v. 31 hours, $p = 0.035$).
- Abnormal HR, temperature, and spO₂ alone did not correlate with LOS.
- When controlling for temperature, there was no significant difference in LOS for patients with pre-transfer abnormal HRs.
- Younger patients were less likely to have a recorded BP ($p < 0.001$), with a 10.75 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

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

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Abstract

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

Materials and Methods

Culture and Differentiation 3T3-L1 model of adipogenesis

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

Methionine Concentration Variations in Media

To make medium with varying concentrations of methionine, DMEM/F12 and DMEM high glucose with no methionine was ordered (Gibco). Dialyzed FBS was used to ensure no added methionine was present. Induction and maintenance medium was made using methionine 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

Results

3T3-L1 model of adipocyte differentiation

Figure 1. Adipogenic differentiation of 3T3-L1 cells

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

Effects of methionine modification on adipocyte cell number and differentiation

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.

Acknowledgements and sources of support

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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

Characterizing the pluripotency of human thoracic perivascular adipose tissue progenitor cells

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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 success of adipogenic induction, Alizarin Red for osteogenic induction, and Masson's trichrome for chondrogenic induction. This preliminary work seems to suggest that these human primary PVAT cells are capable of adipogenic and chondrogenic differentiation, but incapable of induction toward an osteogenic lineage.

Background

Perivascular adipose tissue, or PVAT, is a specialized form of fat tissue that encompasses major vasculature in the body. While this tissue was once thought to be primarily structural in nature, PVAT is now understood to play a significant role in regulating vascular health. In metabolically healthy individuals, PVAT both promotes vasodilation and inhibits inflammation. In metabolically unhealthy individuals, as in conditions of obesity, PVAT expands through a process called hypertrophy and loses its protective functions, exacerbating the risk of cardiovascular disease. Since 2016, the Liaw lab at the MMCRI's Center for Molecular Medicine has made the study of perivascular adipose tissue a primary focus of its work.

Coronary artery bypass graft (CABG)

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 explanation of primary cells.

Human PVAT-APC differentiate to adipocytes *in vitro* a) Oil red O (ORO) staining of lipids in non-induced (top) and induced (bottom) at day 7. b) Immunoblot for the indicated proteins of non-induced and induced PVAT-APC for 7 and 14 days.

The Liaw lab's Joshua Boucher, PhD, has been able to explant progenitor cells from these human PVAT samples and successfully induce them to differentiate toward an adipogenic lineage. The success of differentiation was assessed by staining for neutral lipid accumulation with Oil Red O as well as probing for expression of genes specific to mature adipocytes, such as *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

Adipogenic

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

Osteogenic

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

Chondrogenic

Fig. 3. In the chondrogenic induction assay, shown in these images, human thoracic PVAT cells were cultured using a "micromass" technique which allows for the growth of a pellet of roughly 10⁷ cells in the center of a culture dish. These cells were cultured in chondrogenic differentiation media for 14 days and then the cell pellet was embedded in paraffin and stained using Masson's trichrome, of which the blue stains for collagen. In multiple pellets of human thoracic PVAT cells,

Neoadjuvant Therapy versus Upfront Surgery for Resectable Pancreatic Cancer using the NCDB database: A Decision Analysis

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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, T1NXM0, T2N0M0, T2N1M0, T2NXM0
 - Patients undergoing palliative care were excluded
 - Patients who refused surgery were excluded
- Descriptive statistics were used analyze the data

References

- Cronin KA, Lake AJ, Scott S, et al. Annual Report to the Nation on the Status of Cancer, part I: National cancer statistics. *Cancer*. 2018;124(13):2785-2800.
- 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):588-594.
- de Groot 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.
- 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.

Kaplan-Meier Survival Curves

Variable	HR (95% CI)	P value
Age, 65-79	1.15 (1.09-1.21)	<0.0001
Age, 90-99	1.62 (1.58-1.77)	<0.0001
Male	1.05 (1.01-1.08)	0.004
White	1.02 (0.97-1.07)	0.439
Medicare	1.02 (0.96-1.08)	0.551
Private insurance	0.88 (0.83-0.93)	<0.0001
Academic center	0.79 (0.77-0.82)	<0.0001
Metropolitan area	0.92 (0.88-0.95)	<0.0001
Comorbidities	1.15 (1.11-1.18)	<0.0001
Poor tumor grade	1.26 (1.21-1.31)	<0.0001
Head/Neck Tumor	1.09 (1.06-1.14)	<0.0001
Start with Surgery	0.48 (0.46-0.49)	<0.0001

Decision Tree

Conclusions

- 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.

Next Steps

- 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

Summer Student Research Program (SSRP)

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INTRODUCTION

- Lysosomal acid lipase (LAL) is an enzyme in the lysosome that breaks down cholesteryl esters (CE) and triacylglycerides (TAGs).¹
- In humans, an LAL deficiency presents itself in two diseases:
 - Wolman Disease -, infants experience severe hepatosplenomegaly and malabsorption and usually die before one year of age
 - Cholesteryl ester storage disease - later onset and milder phenotype, resulting in CE storage in visceral tissues, hepatosplenomegaly, and hypercholesterolemia.¹
- LAL knockout (KO) mice have increased TAG and CE storage in various tissues², as well as defective brown adipose tissue, leading to hypothermia at room temperature.³
- Recently, our lab has demonstrated LAL KO mice also have decreased bone volume/ total volume (BV/TV) in the distal femur metaphysis.

Adapted from Hong Dc, Journal of Lipid Research, 2001

Unpublished data.

SCHEMATIC DEPICTING LAL'S ROLE

"Normal" LAL

LAL Deficiency

HYPOTHESIS AND EXPECTED OUTCOMES

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

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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 5.0×10^5 cells per well in three 12-well plates.

Lalstat Treatment

- After 48 hours, the alpha MEM complete medium was replaced with osteogenic medium containing 50 µg/mL ascorbic acid and 5 mM β-glycerolphosphate. The cells received this differentiation medium for seven days.
- Throughout the differentiation, cells received various doses of Lalstat at either 0 µM (Control), 25 µM, 50 µM, or 100 µM. 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 LALSTAT 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 Lalstat group of bone marrow stromal cells

CONCLUSIONS & SUMMARY

- ALP stains showed mixed results amongst the three plates, making the results inconclusive even though the photographs showed less confluence in the Lalstat-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.

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) Datta-Mare, M. (2018). Lysosomal acid lipase regulates fatty acid channeling in brown adipose tissue to maintain thermogenesis. *BBA - Molecular and Cell Biology of Lipids*.

Predicting Neurological Outcomes Using Pupillometry During Targeted Temperature Management After A Cardiac Arrest

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Abstract

A pupil's reactivity to light during targeted temperature management (TTM) after a cardiac arrest can help predict the neurological outcome of a patient.¹ This was investigated by examining pupillometer data from patients admitted to Maine Medical Center from August 2017 - July 2018. Neurological Pupil Index (NPI), percent constriction and pupil size were compared to previously-established thresholds^{1,2}, while this is the first study, to our knowledge, to establish a threshold for constriction velocity (via ROC curve). This data was conducted in order to find trends consistent with good/poor neurological outcomes in patients who suffer from cardiac arrests. A NeuroOptics NPI-200 pupillometer was used by nurses every few hours (no specific time requirement) both during and after TTM in order to get objective pupil exams. Comparison of pupillometer data was done by using patient's worst scoring eye (in each respective category) between Good or Poor outcomes and statistically analyzed by using Mann-Whitney, Chi-Squared, and Fisher Exact tests. This study supports that NPI (≥ 0 and/or < 3), percent constriction ($< 13\%$), and constriction velocity ($< 0.54\text{mm/sec}$ and $< 0.32\text{mm/sec}$) at different time periods (< 6 hours after ROSC, < 24 hours after ROSC, or ever) are good predictors in neurological outcomes of patients after a cardiac arrest.

Introduction

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

*When a person has a cardiac arrest (CA), significant brain damage can result due to lack of oxygen to the brain. Targeted temperature management (TTM) has been demonstrated to reduce the amount of brain damage that occurs in these patients.³

*The goal of this study was to examine data from the pupillometer (specifically Neurological Pupil Index (NPI), percent constriction, constriction velocity, and pupil size) and compare the values to suggested thresholds^{1,2} in order to predict a person's neurological outcome. We did this by looking at three time intervals: 6 hours after return of spontaneous circulation (ROSC), 24 hours after ROSC, and ever.

*Our hypothesis was that constriction velocity would be a more accurate predictor of neurological outcome than NPI and size.

Patient Variables vs Outcome (n=61)			
Age, years Median (IQR)	60 (55-66)	p=0.2530	
Male, n (%)	42 (69%)	p=0.8875	
Initial Rhythm		p=0.0001	
VT/VF/AED shockable	24 (39%)		
Asystole	15 (25%)		
PEA	14 (23%)		
Unknown	8 (13%)		
Witnessed Arrest	42 (69%)	p=0.1400	
Time to ROSC, minutes	15 (8-25)	p=0.5752	
DOHCA	46 (75%)	p=0.5751	
Good Outcome (CPC 1-2)	17 (28%)		

	Good Outcome	Poor Outcome	p-value
NPI	N=17 (28%)	N=44 (72%)	
<6hrs	4.4 (3.7-4.5)	3.5 (1.8-4.1)	0.0029
<24hrs	3.6 (2.2-4.1)	2.8 (0.4-3.6)	0.0139
Total	3.6 (2.2-4)	1.8 (0.3-3.5)	0.0036
Size (mm)			
<6hrs	2.59 (2.15-3.86)	2.67 (2.24-4.24)	0.5464
<24hrs	3.86 (2.61-4.55)	3.5 (2.44-5.16)	0.5912
Total	4.22 (3.29-5.11)	4.43 (3.09-6)	0.6578
Constriction Velocity			
<6hrs	0.81 (0.65-0.96)	0.34 (0.18-0.53)	0.0002
<24hrs	0.44 (0.25-0.51)	0.18 (0.1-0.29)	0.0027
Total	0.44 (0.27-0.5)	0.15 (0.08-0.28)	0.0005
% Constriction			
<6hrs	16 (13-19)	9 (4-13)	0.0002
<24hrs	8 (7-12)	4 (2-7)	0.0005
Total	8 (7-12)	4 (2-6)	<0.0001

*Npi=0: 0 of 7 (0%) Good Outcome (n=7; p=0.0934)

*0<Npi<3: 2 of 12 (17%) Good Outcome (n=12; p=0.3219)

*Percent Constriction Bilaterally <13%: 8 of 38 (17%) Good Outcome (n=46; p=0.0003)

*Size >5mm: 5 of 16 (24%) Good Outcome (n=21; p=0.8415)

Results

ROC Curve CV at 6 Hours

ROC Curves NPI, CV, %C at 6 Hours

Methods

<https://neurooptics.com/npi-200-pupillometer/>

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

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

Conclusions

- Constriction velocity was an accurate predictor of poor neurological outcome.
 - Constriction velocity $< 0.54\text{mm/sec}$ within 6 hours after ROSC associated with poor outcome.
 - Constriction velocity $< 0.32\text{mm/sec}$ 6+ hours after ROSC associated with poor outcome.
- NPI value < 3 confirmed as an accurate predictor of poor neurological outcome.¹
- Percent constriction $< 13\%$ confirmed as an accurate predictor of poor neurological outcome.²
- Pupil size confirmed as not an accurate predictor of poor neurological outcome.

Next Steps

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

Acknowledgements

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References

- Shoyombo et al. Understanding the Relationship Between the Neurological Pupil Index and Constriction Velocity Values. Scientific Reports. 2018;8(6992):1-6.
- Selvi et al. Early Prediction of Poor Neurological Outcome After Cardiac Arrest with Dilated

Electrical Cardioversion of Emergency Department Patients with Atrial Fibrillation

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ABSTRACT

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

INTRODUCTION

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

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

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

HYPOTHESIS

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

METHODS

Figure 1: Electrical cardioversion paddle placement on patient with atrial fibrillation

Figure 2: Sinus rhythm displayed on an EKG during 1) atrial fibrillation, 2) during cardioversion shock, and 3) during return to normal sinus rhythm

<https://www.hrsonline.org/Patient-Resources/Treatment/Cardioversion>

RESULTS

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

Complication	Percentage of patients
HR < 50 bpm	3.4 %
Ventricular Tachycardia	1.8 %
Systolic BP < 90 mmHg	9.2 %
O2 saturation < 90%	4.0 %
Intubation/Bagged	0.7 %

RESULTS

Figure 3: Average ED visit duration for those with attempted chemical cardioversion versus those utilizing only electrical cardioversion

Figure 4: Number of ED patients returning to the ED within 30 days for atrial fibrillation or related event (n = 680)

CONCLUSIONS

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

REFERENCES

- Burton J, Vinson D, Drummond K, Strout T, Thode H, McInturf J. Electrical Cardioversion of Emergency Department Patients with Atrial Fibrillation. *Ann Emerg Med.* 2004;44(1):20-30.
- DeMeester S, Hess R, Hubbard B, LeClerc K, Ferraro J, Albright J. Implementation of a Novel Algorithm to Decrease Unnecessary Hospitalizations in Patients Presenting to a Community Emergency Department With Atrial Fibrillation. *Continuing Medical Education in Academic Emergency Medicine.* 2018;25(6):642-649.
- Vacarescu C, Petrescu L, Cozma D, et al. Cardioversion of symptomatic persistent atrial fibrillation in patients with left atrium dilation; how far should we go? *Romanian Journal of Cardiology.* December 2017; 27(4): 507-511. Available from: Academic Search Complete, Ipswich, MA. Accessed June 20, 2018.

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Characterization of exosomes secreted from human thermogenic adipocytes

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Abstract

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

Background

During weight gain, periods of prolonged overeating result in fat storage in white adipocytes, which can lead to inflammation, cellular stress, insulin resistance and eventually T2D. Unlike the energy storage function of WAT, brown adipocytes become metabolically activated in response to cold and burn calories by releasing energy stored in fats to generate heat, thereby providing mammals with protection from hypothermia. Therapeutic strategies are being sought to stimulate brown adipocytes to burn excess calories and combat obesity.

(A) Factors contained within exosomes secreted by brown adipocytes may target and promote anti-diabetic effects on tissues affected by type 2 diabetes, such as the liver and white adipose tissue. (B) Exosomes carry a diverse array of signaling molecules, including microRNAs, proteins and lipids that are transported to recipient cells and tissues, potentially through direct homing via specific cell surface receptors. (C) microRNAs carried by exosomes can alter gene expression in distant tissues by binding to specific mRNA targets and silencing them.

Hypothesis

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

Methods

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

Results

Isolation of exosomes from brown adipocytes

Exosomes were isolated from 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 3000xg to remove cell debris and was then combined with 2ml of Total Exosome Isolation reagent and incubated overnight at 4°C. Exosomes were isolated by centrifugation at 10,000xg 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 CD81.

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, including miRNA-92a, miRNA-99b and U6 snRNA. U6 snRNA is a non-coding small nuclear RNA commonly used as internal control to normalize microRNA expression in cells. cDNA was amplified by qPCR using TaqMan probe based assays for these 3 targets. These data suggest that we were able to successfully isolate microRNAs from the exosomes of brown adipocytes.

miRNA-32 is enriched in exosomes compared to brown adipocytes

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

Summary

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

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The Role of Lipid Metabolism in Multiple Myeloma

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INTRODUCTION

- **Multiple Myeloma (MM)** is the second most common type of hematological cancer, formed from a series of oncogenic mutations to the plasma cells of the bone marrow (1).
- Initially patients respond well to chemotherapeutic treatment, but almost all eventually develop resistance to these treatments and experience relapse.
- Myeloma thrives in the unique and complex bone marrow microenvironment. Also, within the bone marrow are bone marrow adipocytes (BMA) that form bone marrow adipose tissue and account for 50-70% of the total bone marrow volume. It is believed that BMAT provides a source of energy that aids in multiple myeloma cell metastasis (2).
- Fatty acid oxidation is the process by which cells convert long chain fatty acids into NADH, FADH₂, and ATP in the mitochondria.
- CPT1 is a transport enzyme in the outer mitochondrial membrane that transports long chain fatty acids into the inner mitochondrial space. It is the first, yet rate limiting enzyme of the carnitine system and subsequently of fatty acid oxidation (3).
- Etomoxir (Eto) is 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.

Figure 1 : Melone et al. *Cell Death & Disease* 2018. The carnitine system processes long-chain fatty acids across the mitochondrial membrane, forming acylcarnitine. Acylcarnitine then enters the mitochondrial matrix via carnitine-acylcarnitine translocase (CACT) to undergo β -oxidation. Once in the form of Acetyl CoA, the fatty acid enters the Krebs cycle to produce ATP. (6)

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. Bortezomib and dexamethasone are common pharmacological anti-myeloma treatments.

Cell Number: Bioluminescent imaging (BLI) was used to quantify cell viability. Luciferin (10 μ L) 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

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RESULTS

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

Cell viability of (A) OPM2 and (B) MM1R cells treated with etomoxir (eto) at 0 μ M, 5 μ M or 12.5 μ M, 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. *, $p < 0.05$; **, $p < 0.01$.

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 (5 μ M) and dexamethasone (0.5 μ M) 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