Summer Programs Symposium

August 3rd – August 4th, 2022

Abstract Booklet

Summer Program for Undergraduate Research (SPUR)

Gokul Pareek – University of Illinois Chicago

Jing Dong, PhD

Division of Hematology and Oncology, Department of Medicine

MCW Cancer Center

Linda T. and John A. Mellows Center for Genomic Sciences and Precision Medicine

---

A Genome-wide Association Study of Multiple Myeloma and its Precursors

Background

Multiple myeloma (MM) is a rare form of cancer characterized by disproportionate proliferation and impaired functioning of plasma cells found in the bone marrow. However, MM typically occurs as a comparatively benign disease of monoclonal gammopathy of undetermined significance (MGUS), followed by smoldering multiple myeloma (SMM), and gradually progressing to MM. To identify genetic determinants of MM and its precursors, a genome-wide association study (GWAS) is performed with a focus on associations between single-nucleotide polymorphisms (SNPs), including Human Leukocyte Antigen (HLA) alleles, and disease specific traits.

Method

We used the Illumina Infinium Global Screening Array to scan 264 MGUS and 649 MM patients enrolled from the Medical College of Wisconsin (MCW) Tissue Bank. After a standard quality control (QC), 249 MGUS and 619 MM patients were included in the analysis (*Table 1*). We used IMPUTE2 to impute missing SNPs using the 1000 Genomes Phase 3 as a reference panel, and HLA*IMP:03 pipeline was used to impute HLA alleles. Publicly available dataset retrieved through the Database of Genotype and Phenotype (dbGaP) will be manipulated using the same methods and meta-analyzed with our data. A post-imputation QC was conducted to remove variants with low imputation quality score ($R^2 < 0.3$). Logistic regression analysis was performed using PLINK with adjustment of age, race, sex, and top principal components.

Results

Through this study, I have improved my knowledge in molecular epidemiology and gained hands-on experiences in 1) QC for GWAS and dbGaP data; 2) EIGENSOFT package for principal components analysis; and 3) HLA imputation and data analysis. Association analysis for HLA alleles showed that eight classical HLA alleles were associated with the progression from MGUS to MM with *P*-values < 0.05 (*Table 2*). The preliminary data has resulted in an abstract submitted to the 64th Annual Meeting of the American Society of Hematology (ASH), and a manuscript in preparation, both of which I serve as a co-first author. Since additional analysis focusing on the risks of MGUS and MM, and MM prognosis are ongoing, I would continue to work with my mentor on the GWAS study after the SPUR and would serve as a co-author on the manuscripts derived from the same.

Conclusion

The results of the study indicate that HLA alleles may contribute to the progression of MGUS to MM.

Keywords: Genome-wide Association Study; Multiple Myeloma; HLA

			Gender		Age		
		Total	**Male (%)**	**Female (%)**	**Median**	**Low**	**High**
White	**MM**	552	62.4	37.6	62.6	22.2	94.8
	MGUS	204	44.7	55.3	67.6	28.8	95.5
Black	**MM**	63	50.8	49.2	58.9	32.3	78.2
	MGUS	42	38.1	61.9	64.5	41.9	79.3
Others	**MM**	4	50	50	56.7	40.5	71.3
	MGUS	3	33.3	66.7	69.9	52.7	78.6

Table 1: Demographics of MGUS and MM patients.

HLA Locus	**Variants**	**OR (95%CI)**	***P-value***
HLA_DRB5	99:01	4.32 (1.56-11.92)	4.79E-03
HLA_A	30:01	5.49 (1.53-19.71)	9.03E-03
*HLA_B**	08:01	0.43 (0.21-0.86)	1.69E-02
HLA_DRB1	15:03	0.32 (0.13-0.83)	1.89E-02
HLA_A	30:02	0.37 (0.16-0.85)	1.96E-02
HLA_DQA1	01:03	1.68 (1.03-2.77)	3.97E-02
*HLA_C**	07:01	0.63 (0.41-0.98)	4.17E-02
HLA_DRB1	04:02	8.87 (1.00-78.37)	4.97E-02

Table 2: Imputed HLA allele with odds ratio for developing MM compared to MGUS.

Signe Penn – Bethel University
Ramani Ramchandran, PhD
MCW Department of Pediatrics

Bardet-Biedl Syndrome: a new gene candidate?

Background

Bardet-Biedl Syndrome (BBS) is a rare autosomal recessive ciliopathy with pleiotropic effects. Symptoms of BBS include obesity, renal dysfunction, polydactyly, and learning difficulties. Studies of BBS have identified at least 20 genes that can account for 80% of clinically diagnosed cases. More research is necessary to discover the genetic mutations responsible for the remaining 20% of BBS cases. Uncovering the remaining genetic mutations responsible for BBS will improve diagnosis and treatments of this disease. Prior unpublished research has discovered a mutation in a novel gene in patients who lack any of the mutations known to cause BBS but exhibit common symptoms. The novel genetic mutation identified is in the sucrose nonfermenting 1-related kinase gene, which codes for sucrose nonfermenting 1-related kinase (SNRK). The mutation is a single nucleotide substitution that results in the amino acid mutation P388L. The overall goal of this project is to determine if the P388L mutation in SNRK is responsible for the BBS phenotype. To begin, we hypothesized the P388L mutation in SNRK will alter normal protein function.

Method

To investigate this hypothesis, we overexpressed the mutant protein *in vitro* in primary human brain microvascular endothelial cells (HBMVECs) and compared it to over expressed wild type protein. SNRK proteins were expressed in HBMVECs using lentiviral transduction method. Data was then gathered on protein expression, localization, and proliferation using Western Blot, immunofluorescence, and flow cytometry, respectively. Each experiment was repeated two to three times.

Results

Results were collected in graphical and pictorial format. Preliminary results gathered from these experiments indicate the expression levels of SNRK impacts cell morphology and proliferation and suggest there is indeed a difference in function between wild type and mutated SNRK.

Conclusion

The next steps involve testing this hypothesis *in vivo*. These approaches will conclusively determine the effect of the P388L mutation on SNRK function. This information will allow for the addition of SNRK to the database of BBS mutations, providing another resource to use in diagnosis of BBS and close the gap of the 20% of undiagnosed BBS cases.

Isabelle Grassl – Marquette University
Dawn Wolfgram, MD
MCW Department of Nephrology

Cerebrovascular Reactivity in Hemodialysis Patients

Background

Patients with end-stage renal disease treated with hemodialysis (HD) have significant cerebral ischemic disease on neuroimaging. This injury may be related to HD treatment, as changes in cerebral perfusion can occur due to the significant fluctuations in blood pressure (BP) during HD, a process that is normally controlled through cerebral autoregulation. We hypothesize that patient who experience cerebral hypoperfusion during HD have impaired cerebral autoregulation. In this study we measure cerebrovascular reactivity (CVR), as a marker of cerebral autoregulation, and determine the relationship to cerebral hypoperfusion during HD.

Method

We used Transcranial Doppler (TCD) to measure CVR and cerebral oximetry to measure changes in cerebral perfusion. For the CVR, we recorded the changes in mean flow velocity of the middle cerebral artery in response to a 30 second breath hold for three trials, with the percent change in velocity per mmHg in end-tidal CO2 calculated for CVR, lower value indicating worse CVR. For the cerebral oximetry procedure, we used forehead sensors to measure the patient's cerebral oxygen saturation (ScO2) during their entire HD session and calculated the drop in ScO2 as the beginning ScO2 minus the minimum ScO2 recorded during the session. We also calculated a cerebral autoregulation index (CA index) by obtaining the correlation between concurrent BP and ScO2 values during the HD session. We will use linear regression and correlation analysis to examine the relationship between CVR and change in ScO2 during HD and CVR and CA index.

Results

There are currently fourteen participants enrolled in the study. The mean age of the participants is 62 (with a standard deviation of 10.7) years, with 60% having diabetes and 100% having hypertension. We started study procedures on 6 participants, but two were unable to complete the TCD and one has the cerebral oximetry pending. Thus, we have three participants who have completed the study procedures. For these three participants, the average CVR from the first trial is 4.10% (right) and 4.00% (left). The average CA index was 0.20 (right) and 0.22 (left). The average drop in ScO2 during HD was 8.2%.

Conclusion

Data collection is ongoing, but successfully measures CVR and CA index in HD participants. We note that cerebral perfusion measured through cerebral oximetry does decline during the HD session indicating cerebral hypoperfusion. Our results will determine if CVR does correlate with the amount of decline in ScO2 during HD.

Kai Liptow – Ripon College
Elena Semina, PhD
CRI Department of Pediatrics - Developmental Biology

A Functional Analysis of *PITX2* and *KMT2D* Intronic Variants

Background

The human eye is a complex organ composed of specialized tissues which develop through the highly coordinated process of cell differentiation. Genetic or environmental components that alter the development process or any of its parts can lead to a variety of developmental ocular disorders. While genetic variants affecting the coding regions of genes are often easier to mechanistically explain, variants affecting non-coding DNA are more difficult to identify as causative factors in developmental disorders. Two such variants were identified by analysis of patient exomes within the *PITX2* and *KMT2D* genes. One mechanism by which non-coding variants may alter function is through cryptic splicing, or splicing resulting in the insertion of a nonfunctional intronic sequence within the mature mRNA transcript. The purpose of this study was to determine any splicing effects unique to the patient variants identified in the *PITX2* and *KMT2D* genes.

Methods

Variants of interest were identified through *in silico* analysis programs, VarSeq, SpliceAI, and the UCSC Genome Browser, based on predicted cryptic splicing effects and conservation. To determine any disadvantageous effects caused by these predicted splicing variants, B3 lens epithelial cells were transfected with wildtype and variant sequences for both genes of interest and monitored for splicing variation. To create the constructs for transfection, the genomic segment of *KMT2D* containing the variant was amplified from control DNA and cloned into V5-His-TOPO TA vector; variant construct was developed by mutagenesis of the wild-type sequence. For *PITX2*, these steps were bypassed using gBlocks, or synthetic double stranded DNA segments generated by IDT, which were directly cloned into V5-His-TOPO TA vector for use in transfection.

Results

A 1700-bp wild-type genomic fragment of *KMT2D* was successfully amplified using high-fidelity Taq polymerase, gel purified, and cloned into V5-His-TOPO TA vector. Four positive colonies were selected based on colony PCR and DNA was submitted for sequencing. 1046-bp gBlocks for wild-type and variant *PITX2* were obtained from IDT, cloned in V5-His-TOPO TA vector, and five wild-type and two variant clones were submitted for sequencing. Following sequence verification, transfections will be performed, and results will be presented.

Conclusion

A more comprehensive understanding of the genetic mechanisms underlying developmental ocular disorders inherently opens the door for new and improved treatment and serves to provide afflicted patients with more diverse treatment options. Furthermore, improving understanding of intronic variants and their effects stands to improve the genetic analysis of patients. Further work is needed to better predict the effects of non-coding variants.

Thomas Gonzales – Vanderbilt University
Dr. Xiaowen Bai, MD, PhD
MCW Department of Cell Biology, Neurobiology and Anatomy (CBNA)

Changes in mitochondrial dynamic due to alcohol induced-neurotoxicity in human stem cell derived neurons.

Background

Prenatal exposure to alcohol can have significant impacts on mental and physical development, known as fetal alcohol spectrum disorder (FASD). The disruption of normal cognitive development can result in many significant complications for the child and costs billions of dollars in related spending annually. Yet despite the significance of the disorders, little is known about how alcohol affects the human prenatal brain. The goal of this study is to use human induced pluripotent stem cell (iPSC)-derived to study the toxic effect of alcohol (ethanol) on the mitochondrial dynamic (fission and fusion) within neurons and examine whether Mdivi-1 (a mitochondrial division inhibitor) or brain derived neurotrophic factor (BDNF) can reduce the toxic effect of the ethanol. Imbalances in the mitochondrial fission and fusion dynamic could result in neurodegeneration and many other pathological issues and as such are of particular interest. The main hypothesis of this experiment is that the neurons exposed to ethanol will exhibit an imbalance in mitochondrial dynamic resulting in fragmentation, but those exposed to Mdivi-1, and BDNF will experience an attenuated response.

Methods

2-dimensional neurons were differentiated from human iPSC cultures. Neurons were then exposed to 50 mM of ethanol for 6 hours to simulate the level of alcohol a fetus would be exposed to during a binge drinking episode equivalent to blood ethanol concentration of the mother consuming between 5 and 6 standard drinks depending on body weight. To study the neuroprotective effect of Mdivi-1 and BDNF, neurons were either pretreated with Mdivi-1 for 30 minutes prior to ethanol exposure or treated with BDNF and ethanol for 6 hours. The cells were then fixed and immunostained with TOM20, a mitochondrial marker, in order visualize the mitochondrial shape and observe changes in aspect ratio (AR) and form factor (FF) of the mitochondria, measurements which can be used as indicators of the mitochondrial dynamic.

Results

The Mitochondrial stains showed that there was a change in mitochondrial morphology observed between the control and ethanol exposed neurons. Both AR and FF were significantly reduced when exposed to ethanol, indicating an imbalance in mitochondrial dynamic. The Mdivi-1 and BNDF exposed groups significantly diminished this reduction.

Conclusion

The study showed that ethanol induced neurotoxicity resulting from binge drinking and caused changes in neuronal mitochondrial morphology shifting the balance from mitochondrial fusion to fission. The Mdvi-1 attenuated ethanol-induced changes in mitochondrial morphology, and the mechanism by which BDNF attenuated the fragmentation warrants a more detailed study.

Asad Akhter University of Wisconsin Parkside

Nathan Schloemer, MD

MCW Department of Pediatrics. Division: Hematology/-Oncology/Transplant

Osteosarcoma Mass Spectrometry Immunotherapy Target Identification

Osteosarcoma is the most common bone tumor in pediatrics, comprising of 2-3% of childhood cancers. Despite intensification of treatments the outcomes for osteosarcoma remain dismal. Novel therapies are required to improve survival. Immunotherapy, harnessing the power of the immune system to treat cancer, is a promising novel approach. Specific surface targets are required to generate antibody mediated immunotherapies against tumor cells. We performed mass spectrometry to identify the expressed proteins of multiple osteosarcoma cell lines. To identify the ideal targets for immunotherapy development we performed multiple analyses. First, we selected proteins with consistent high expressions in tumor cells. Next, using the SurfaceGenie tool, we selected unique proteins displayed on the cell surface. Finally, using The Human Protein Atlas, we sorted these proteins into groups based on tumor expression with minimal normal tissue expression. From an initial dataset of 3254 we identified 11 ideal targets to guide novel immunotherapy development. In those 11 identified targets, 7 have already been explored as cancer directed therapy targets but 4 represent novel findings. In conclusion, we have identified potential targets for novel osteosarcoma immunotherapy development. We will next verify our proteomic findings by assaying existing patient samples followed by manufacturing antibody mediated immunotherapies.

Nour Chaer[1], Charles E Hay[2], Mary Faber[2], William Mckillop[2], Jeffrey A. Medin[2,3]

[1]University of Wisconsin-Parkside, [2]MCW, Department of Pediatrics, [3]Department of Biochemistry

---

Development of a Novel Anti-Lymphoma Bispecific Antibody

Background and Significance:

Hodgkin's Lymphoma, HL, is a cancer of the lymphatic system that starts in lymphocytes and results in their uncontrolled growth. About 9,000 people in the United States are diagnosed with HL each year. Currently, there is an 88% survival rate at five years. This number, because it is not 100%, emphasizes the need to develop new therapies to treat lymphoma. Immunotherapy is an emerging field of treatment that helps a patient's own immune system recognize and target cancer cells. Bispecific antibodies are a type of immunotherapy that can bind two different antigens or epitopes. Bispecific antibodies have fewer off-target side effects compared to chemotherapy. The Medin lab has developed a novel bispecific antibody, which can bind to lymphoma cells on one end and to T cells on the other end, enabling the T cells to recognize and lyse HL cells.

Methods:

DNA coding for the novel fusion antibody sequence was inserted into pcDNA 3.4 plasmids. These plasmids were transformed into *E. coli* and later harvested via giga preps. Next, the plasmids were transfected into Expi293 cells, which expressed and secreted the antibody. The antibody was then harvested from the cell supernatant after seven days. Coomassie stains and Western blots were used to characterize the proteins. Flow cytometry was used to show T cell recruitment to specific tumor-associated antigen positive (TAA+) cells.

Results:

Coomassie staining and Western blots confirmed that the antibody was expressed and was approximately the correct size. Flow cytometry results show that T cells armed with the antibody are able to recognize and bind

TAA+ cells. Furthermore, the flow results provided evidence that the armed T cells were beginning to lyse the TAA+ cells after one hour.

Summary and conclusion:

Our results show that our unique anti-lymphoma bispecific antibody is expressed and can bind T cells and TAA+ cells, which allow the T cells to lyse them. These initial results show that our anti-lymphoma antibodies show promise as a potential therapy to treat Hodgkin's lymphoma.

Keywords:

Keywords: Immunotherapy, Bispecific Antibody, T-cells, Lymphoma

Cassius Guthrie – University Of North Carolina Asheville
Ayana Jamal, MSTP
MCW Department of Cell Biology, Neurobiology, and Anatomy

Establishing a Baseline Response to Chronic Isoproterenol Exposure in Larval Shroom3 *Danio rerio*

Background

A myocardial infarction (MI), or heart attack, occurs when areas of the cardiac muscle fail to receive oxygenated blood, resulting in tissue death. While the damaged area is replaced by fibrotic scar, this leads to decreased cardiac function that ultimately progresses to heart failure (HF)[4]. The Zebrafish (*Danio rerio*) is a popular model to study cardiac dysfunction due to its extensive regenerative capabilities. This study seeks to use the pharmacological agent isoproterenol (ISO) to induce heart failure in zebrafish larvae as a model to examine the pathological role of various genes including *Shroom3*. *Shroom3* codes for a protein hypothesized to localize at adherens junctions where it plays a role in determining cardiomyocyte shape and polarization[1]. The goal of this investigation is to determine the baseline response in this genetic model that can be used as a standard to study cardiac HF and potential targets for pro-regenerative therapeutics.

Method

All animal handling and experimentation were performed in accordance with guidelines set by the NIH.

Beginning at 3 days post-fertilization (dpf), *Shroom3* wildtypes and mutants were transferred to petri dishes containing a solution of 1mM ISO diluted in Instant Ocean water with phenyl-thiouria to prevent pigmentation. The solution was prepared and changed daily for four days, and larvae were kept in a 28ºC incubator[2]. At the end of the treatment (7dpf), five fish of each genotype from each birth cohort were immobilized in 2% agarose to record heart function using a Nikon video microscope. This procedure was repeated with untreated larvae as controls. Once recorded, larvae were fixed in 4% paraformaldehyde for immunostaining.

ImageJ was used for quantification of larval heart function. For each sample, three frames of the heart in systole and diastole were selected to measure heart length, width, and area. Statistical analysis was done using RStudio[3].

Results

There is no significant difference between the ejection fraction of *Shroom3* treated and untreated larvae (Student's T-Test, t=-1.2627, df=20.834, p=0.2206).

Conclusion

Initial results suggest that four-day ISO treatment does not affect cardiac function in *Shroom3* larvae. Previous publications indicate that ISO-induced HF is both dosage and duration dependent, yet larvae showed response beginning at 3dpf under the conditions implemented here[2]. Additionally, ISO-treated wild type larvae exhibited high rates of cardiac abnormalities, possibly due to unrelated clutch-specific background. Although preliminary findings show no significance, the study should be repeated with a higher sample size from various birth cohorts and clutches to minimize any variance.

Bibliography

1. Durbin MD, O'Kane J, Lorentz S, Firulli AB, Ware SM. 2020. SHROOM3 is downstream of the planar cell polarity pathway and loss-of-function results in congenital heart defects. Developmental Biology. 464(2):124–136. doi:10.1016/j.ydbio.2020.05.013.

2. Kossack M, Hein S, Juergensen L, Siragusa M, Benz A, Katus HA, Most P, Hassel D. 2017. Induction of cardiac dysfunction in developing and adult zebrafish by chronic isoproterenol stimulation. Journal of Molecular and Cellular Cardiology. 108:95–105. doi:10.1016/j.yjmcc.2017.05.011.

3. RStudio Team. 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston. http://www.rstudio.com/.

4. Sanz-Morejón A, Mercader N. 2020. Recent insights into zebrafish cardiac regeneration. Current Opinion in Genetics & Development. 64:37–43. doi:10.1016/j.gde.2020.05.020.

John Peters – Milwaukee School of Engineering,
Kimani Njoya & Michelle Riehle, PhD
Department of Microbiology and Immunology
Medical College of Wisconsin

Experimental Validation of a Machine Learning Model to Improve Enhancer Prediction

Background

Enhancers are *cis*-acting regulatory elements in the non-coding genome that control regulated gene expression. Since enhancers do not have well-defined sequence patterns, experimental methods are employed to identify enhancers. Two common experimental methods for locating enhancers are chromatin accessibility assays like DNase-Seq and histone modification assays like ChIP-Seq H3k4me1. These high throughput assays identify regions of open chromatin, a subset of which contain enhancers. Machine learning models were trained to distinguish between genome regions identified by those assays (peaks) and those not based on the underlying sequence; it was hypothesized that since enhancer activity is tied to sequence, the models could be used to filter the assay data for enhancers. The goal of this project is to experimentally test the predictions from the machine learning model for enhancer activity using luciferase assays.

Methods

Machine learning models were trained on sequences from open chromatin regions identified by DNase-seq and ChIP-Seq H3k4me1 performed in Drosophila S2 cells and randomly sampled non-coding control sequences. The models were trained and applied in a five-fold cross-fold validation to obtain predictions for every peak sequence. Ten sequences classified as true positives and false positives for enhancer activity (20 total for each method) were randomly sampled. Sequences were experimentally tested for enhancer activity using luciferase assays. Enhancer fragments were PCR amplified from S2 genomic DNA and cloned into an expression vector upstream of a firefly luciferase reporter. Using lipid-based transfection, these plasmids, along with a renilla luciferase reporter plasmid, were transfected into S2 cells at mid-log growth. Twenty-four hours following transfection, luciferase activity was measured using a luminometer. Luciferase activity was used to assess whether the model predictions correlated with enhancer activity.

Results

The machine learning model indicates that DNase-seq predicted enhancers contain a stronger sequence-peak correlation than ChIP-Seq H3K4me1 predicted enhancers. Area under the receiver operator characteristic (ROC) curves from the model's predictions present accuracies of 0.892 and 0.743, respectively. Luciferase assays to validate these predictions are currently ongoing and data on model accuracy will be presented.

Conclusion

Current experimental validation of the model is ongoing. However, with the current difficulty in understanding the non-coding lexicon, there is a significant interest in computational methods equipped to identify enhancers and similar non-coding regulatory elements. In the future, experimentally supported machine learning models may play a significant role in the computational prediction of enhancers and understanding of the non-coding lexicon.

Daniela Avila – Alverno College
Vera Tarakanova, PhD
MCW Department of Microbiology and Immunology

Mimicking the System: Chronic Infection Response to PolyI:C Treatment

Gammaherpesviruses (GHV) are pathogens that establish lifelong infections similar to Epstein Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). They have been linked to cancer development, associated with various B cells lymphomas. GHV has been found to manipulation the germinal center. The virus is able to reactivate after it becomes latent. Their latent reservoir is found in the memory B cells, they will become plasma cells to bring the virus from latent to lytic once again. This study focuses on the host factors that control gammaherpesvirus infection to understand how these viruses cause disease. One of our major antiviral systems is known as type I interferon (IFN). This pathway will produce hundreds of interferon-stimulated genes (ISGs) to protect against a virus. To stimulate the activation of type I IFN system a synthetic double-stranded RNA to injected as a prior infection before GHV is introduced. The establishment of chronic infection at 16 days was developed. In order to show the prior infection that actives type I IFN we injected C57BL/6J mice with polyinosinic-polycytidylic acid (PolyI:C). It is the synthetic equivalent of double stranded RNA that interacts with TLR3 to drive type I IFN production. This mimics the natural dsRNA that is known to activate the immune system. Experiments involved the isolation of three conditional groups; injected at 7 and 3 days prior to MHV68 infection and no injection in the control group. This virus infection was accomplished by pipetting 1,000 PFU intranasally with the isolation of spleens where B cells are more abundant. After processing the viral DNA results suggest that induction of interferons prior to chronic infection of the host promotes gammaherpesvirus. Using flow cytometry, data determinates proviral activity of interferons could be in part due to expansion of germinal center B cells that host the virus. The switch from acute to chronic allows their function to have proviral functions. The mechanisms of the host and the virus regulates the acute and latent infection within the mutations. As the study was a pilot experience the next steps would involve a larger sample and replicated protocol.

Keywords: gammaherpesvirus, chronic infection, B cell responses, type I interferon

Angeles Morales – University of Central Florida
Stephanie Olivier-Van Stichelen, PhD
MCW Department of Biochemistry

Maternal Non-Nutritive Sweetener (NNS) Exposure Deregulates Hepatic Detoxification Transporters in Offspring

Background

Low in calories while producing a sweet taste, Non-nutritive sweeteners (NNS) are a popular substitute for sugar in the United States. Although the effect of NNS exposure in early stages of life have not been explored extensively, there is no regulation of NNS consumption during pregnancy and lactation. We previously demonstrated maternal NNS exposure during pregnancy and lactation altered detoxification pathways of mice pups while whitening their livers.[1] Efflux ATP-binding cassette (ABC) transporters proteins such as P-glycoprotein (Pgp; Abcb1a/b), breast cancer resistance protein (Bcrp), and multidrug resistance-associated protein 2 (Mrp2) are crucial in drug transport and detoxification. While we previously showed that PGP is inhibited by NNS, we wonder if other ABC-transporters are also impacted by NNS, increasing the liver toxicity in NNS exposed pups. Because inhibition of ABC-transporters often results in their transcriptional dysregulation, this study aimed to measure the expression of *Bcrp* and *Mrp2* in pups' livers of mothers fed NNS during pregnancy and lactation.

Method

Pregnant mice were fed a chow diet supplemented or not with NNS at one or two times the accepted daily intake of NNS (Ctrl/ADI1x/ADI2x) of sucralose and acesulfame potassium. NNS diet was administered daily throughout pregnancy and lactation. Pups were sacrificed at day 19. Flash-frozen pups' livers were pulverized into a frozen, homogenous powder and lysed to extract RNA. Samples were quantified and quality checked on agarose gel prior to cDNA preparation. Mouse primers were designed and tested corresponding to the target proteins: *Abcb1a*, *Bcrp*, *Mrp2* and *β-Actin* (*Actb*), used as the control. Gene expression was measured by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Results

Offspring liver from NNS-fed mice showed downregulation in *Abcb1a* expression while *Mrp2* displayed upregulation in expression when compared to the control treatment group. Variability in control mice led to inconclusive results for *Bcrp* expression. Significance could not be calculated in preliminary results, which used only two mice per treatment group.

Conclusion

Our results demonstrated that maternal exposure to NNS lead to deregulation of efflux ABC transporters expression, suggesting that they might act as inhibitors of Mrp2 as shown previously on Pgp. *Mrp2* was upregulated in ADI1x pups. We hypothesize this was to meet detoxification needs following Pgp inhibition. *Abcb1a* was downregulated in ADI1x pups. Chronic exposure to NNS may have led to inhibitory changes to *Abcb1a* transcriptional regulators. While there are evident trends in expression, genetic variability amongst the pups requires an increase in sample size for statistical analysis. Future studies will assess functional inhibition of Mrp2 and Bcrp by NNS *in vitro*.

Keywords: Detoxification, Sucralose, Acesulfame potassium, Bcrp, Mrp2, ABC Transporter

References

[1] Olivier-Van Stichelen, S., Rother, K. I., & Hanover, J. A. (2019). Maternal Exposure to Non-nutritive Sweeteners Impacts Progeny's Metabolism and Microbiome. *Frontiers in microbiology*, *10*, 1360. https://doi.org/10.3389/fmicb.2019.01360

Role of Endothelial Cell Senescence in Estrogen-Induced Microvascular Dysfunction

Carolyn Shult; Gopika SenthilKumar; Boran Katunaric; Henry Bordas-Murphy; Nicole J. Buiter; and Julie K. Freed

Compared to men whose cardiovascular disease (CVD) risk increases linearly as they age, a woman's risk remains low until they transition to menopause, suggesting that the female hormone estrogen (E2) is protective against the development of heart disease. However, CVD risk is also positively associated with the use of exogenous estrogen (e.g. oral contraceptives, hormone replacement therapy). The effect of E2 on the human microcirculation, a vascular bed that when dysfunctional is predictive of poor cardiovascular outcomes, represents a critical knowledge gap. We have previously shown that following administration of an elevated dose of E2 (100nM), human arterioles collected from cis-females and cis-males develop endothelial dysfunction; however the mechanism contributing to dysfunction is unknown. Here, we hypothesize that elevated E2 induces endothelial cell senescence, a phenotypic change characterized by cell cycle arrest that is associated with endothelial dysfunction. To address this question, male and female human umbilical vein endothelial cells (HUVECs) were treated with increasing doses of 17-beta estradiol (E2; 0.5nM to 100nM). Cells were then maintained for 48hrs in low-serum EBM-2 media prior to beta-galactosidase staining, a well-recognized biomarker of senescence. Treatment groups were imaged using 20x bright-field microscopy, and total number of cells with positive beta-galactosidase staining were counted and normalized to total number of cells per image field. One-Way ANOVA with alpha< 0.05 was used to compare between groups. Male and female HUVECs exhibited no change in percent positive cells with 0.5nM E2 treatment (Male 25±11.4% vs 21.5±2.4% vehicle control; n=3 each; female 4.8±2.9% vs 12.0±4.2% vehicle control, n=1). Male HUVECs had significantly higher percentage of positive cells when exposed to higher doses of E2 (1nM, 45.±11.5%; 50nM, 48.1±6.1%; 100nM, 45.5±2.6%; $p<0.05$, n=3). Similar trends were seen in female HUVECs (1nM, 39.5±17.7%; 50nM, 25.7±9.3%; 100nM, 29.3±13.8%; n=1). These results suggest that elevated levels of E2 may cause an increase in endothelial cell senescence and adds mechanistic insight by which exogenous E2 may contribute to elevated CVD risk.

Tesse Okunseri – Yale University

John Mantsch, PhD

MCW Department of Pharmacology and Toxicology

Effect of Cortisol on Motivated Behavior and the Structure of Dendritic Spines in the Prefrontal Cortex

Background

Depression is a prominent mental illness in society with 21 million U.S. adults having had at least one major depressive episode in 2020.[1] Depressive episodes are often preceded by periods of prolonged or significant exposure to stress,[2] which results in increased levels of circulating stress hormones, such as cortisol. Research has demonstrated that exposure to chronic stress and, subsequent increases in circulating stress hormones, results in significant regressive neuroplastic changes,[3] altering the activity of the prefrontal cortex, a region heavily involved in stress-related signaling and, further, the production of motivated behavior. An aim of my project was to study the effects of chronically elevated stress hormones on individual reward-motivation using a pre-clinical rodent model. A second aim was to characterize the overall prelimbic prefrontal cortical synaptic neuroplastic changes resulting from chronic elevations in circulating stress hormones.

Methods

Using a preclinical rodent model, male subjects were implanted with a slow-releasing pellet containing either corticosterone (CORT)) or a placebo (VEH). To assess differences in motivated behavior, subjects were trained using a classic conditioning task, learning to associate visual and physical stimuli with a food reward. This behavior allows assessment of individual propensity toward either the cue (lever) or the reward (food cup), measures of motivated behavior.

The same subjects were used for post-mortem analysis of dendritic spine morphology utilizing a DiOlistic labeling technique combined with confocal microscopy to collect images of fluorescently labeled prelimbic, pyramidal neurons. Images were assessed utilizing a cutting-edge spine recognition software for 1) morphology changes and 2) overall spine density. Comparisons were performed between CORT conditions.

Expected Results

We expect that a chronic elevation in CORT will result in a significant shift in individual cue- approach motivation towards goal-approach motivation, when compared to non-elevated counterparts. Further, we expect to see significant regressive neuroplastic changes in the dendritic spine morphology of pyramidal, prelimbic neurons. This is characterized by both 1) an overall reduction in the number of spines and 2) a shift in overall dendritic morphology.

Conclusion

We anticipate that our findings will provide insight into the neurobiological processes involved in

[1] Institute of Health Metrics and Evaluation. Global Health Data Exchange (GHDx). http://ghdx.healthdata.org/gbd-results-tool?params=gbd-api-2019-permalink/d780dffbe8a381b25e1416884959e88b (Accessed 11 July 2022)

[2] Tafet, G. E., and Nemeroff, C. B. (2016). The links between stress and depression: Psychoneuroendocrinological, genetic, and environmental interactions. The Journal of Neuropsychiatry and Clinical Neurosciences, 28(2), 77–88. https://doi.org/10.1176/appi.neuropsych.15030053

[3] Anderson, R. M., Glanz, R. M., Johnson, S. B., Miller, M. M., Romig-Martin, S. A., & Radley, J. J. (2016). Prolonged corticosterone exposure induces dendritic spine remodeling and attrition in the rat medial prefrontal cortex. The Journal of comparative neurology, 524(18), 3729–3746. https://doi.org/10.1002/cne.24027

depression and other stress-related disorders, thereby guiding the development of new and more effective treatments.

Lexi Packard[1], Charles E. Hay[2], Mary Faber[2], William McKillop[2], Jeffrey A. Medin[2,3]
[1]University of Wisconsin Parkside, [2]MCW, Department of Pediatrics, [3]Department of Biochemistry

Background and Significance

Multiple myeloma (MM) is a cancer of plasma cells. Normally, plasma cells either circulate in the blood or remain in the lymph nodes. When sufficient mutations occur to the plasma cells to become cancerous, these cells migrate to the bone marrow. Every year nearly 35,000 cases of MM are diagnosed in the United States. The current 5-year survival rate is only 55%. This highlights the importance of developing novel therapies to treat MM.

An emerging field that shows promise to treat MM is immunotherapy. One such therapy utilizes antibodies that recognize two different targets, which are called bispecific antibodies. The Medin lab has designed a bispecific antibody construct capable of binding to a MM antigen, and to T cells. This brings T cells armed with the antibody in close proximity with the target cells and bind to them.

Methods

DNA sequences coding for the anti-MM bispecific antibody were placed in pcDNA 3.4 TOPO plasmids. Plasmids were transformed into competent XL-1 Gold E. coli cells. The plasmids were then harvested via gigapreps. Harvested plasmids were transfected into Expi293 cells. The expressed antibodies were harvested and purified from the cell supernatant after seven days. Coomassie and Western Blots were utilized to characterize the antibodies. Tumor-associated antigen positive (TAA+) cells were dyed with celltrace yellow, while T cells were dyed with celltrace violet. TAA+ cells were incubated with T cells that were armed with our anti-MM bispecific antibodies. The incubated cells were analyzed via flow cytometry to determine if the antibodies can bind both TAA+ cells to T cells.

Results

Coomassie gels and Western Blots (WBs) indicated the anti-MM antibody was expressed at the correct size. Flow cytometry results demonstrated that the bispecific antibody can bind to both TAA+ cells and T cells.

Conclusion

Initial experiments suggest that the expressed bispecific antibody armed T cells can bind to TAA+ cells. The ability to bind the T cell and the target cell of the bispecific antibodies shows promise that they may be used as a future immunotherapy treatment for MM, but further testing will be required.

Amelia Podolny – St. Olaf College

William Hughes, PhD & Andreas Beyer, PhD

MCW Cardiovascular Center

Effects of miR-92a manipulation on autophagy in endothelial cells

Introduction

Cardiovascular disease and its risk factors – such as hyperglycemia – can lead to endothelial dysfunction. Endothelial function is a barometer of overall cardiovascular health and is dependent upon adequate production of the vasodilator nitric oxide in response to shear stress. Conversely, endothelial dysfunction, as observed in subjects with coronary artery disease (CAD), produces H_2O_2 in response to shear stress. Regulation of endothelial function is critical in maintaining cardiovascular health. Autophagy is one of many mechanisms that contributes to normal endothelial function, and our preliminary evidence suggests microRNA-92a (miR-92a) may play a similar mechanistic role. While regulation of autophagy is multifaceted, recent evidence has suggested that miR-92a may play a key role in regulation of autophagy in cardiovascular disease. Indeed, inhibition of miR-92a in rats has been shown to decrease apoptosis and improve recovery of endothelial cells after acute myocardial infarction. Whether miR-92a regulates endothelial cell autophagy is unclear. We investigated whether manipulation of miR-92a impacts endothelial cell autophagy and production of H_2O_2 in response to shear stress in health and CAD. We also evaluated the autophagic responses of endothelial cells that have been exposed to a cardiovascular risk factor, hyperglycemia. We hypothesized that activation of miR-92a would reduce autophagy and increase production of H_2O_2 in response to shear stress in adipose resistance arterioles from healthy subjects. We further hypothesized that inhibition of miR-92a would reduce H_2O_2 production in the context of CAD and prevent the reduction of autophagy induced by hyperglycemia.

Methods

Human adipose arterioles from healthy subjects and those with CAD were isolated and perfused overnight (16-20 h) with miR-92a mimic (miR-92a activation) and α-miR-92a (miR-92a inhibition) to manipulate miR-92a expression. Microvascular redox status was evaluated by shear-induced mitochondrial H_2O_2 production (mitoPY1). Human umbilical vein endothelial cells (HUVECs) were cultured in standard media and split into two groups, one of which was exposed to 20mM glucose. Cells were exposed to manipulation of miR-92a overnight and harvested. Autophagy was measured via western blot by markers LC3B (autophagosome formation) and p62 (autophagolysosome degradation), normalized to GAPDH. Data are expressed as fold-change from control.

Results

Activation of miR-92a in adipose arterioles from healthy subjects demonstrated an increase in shear-induced H_2O_2 production relative to healthy untreated controls (1.69 ±0.41 vs. 0.95±0.07 fold-change, $p < 0.05$; $n = 2 – 7$). Addition of H_2O_2 scavenger, PEG-Cat, did not reduce this increase in H_2O_2 (1.69±0.41 vs. 1.21±0.05 fold change, $p > 0.05$; $n = 2 – 7$). Exposure of arterioles from CAD subjects to α-miR-92a did not reduce shear-induced H_2O_2 production (1.03±0.36 vs. 1.42±0.12 fold change; $p > 0.05$; $n = 2 – 7$). Both inhibition of miR-92a reduced LC3BII and p62 (LC3B fold change: 0.58; p62 fold change: 0.46±0.2; $n = 1 – 2$ experiments), while activation of miR-92a (mimic) also reduced markers of autophagy (LC3B II fold change: 0.92; p62 fold change 0.59±0.01; $n = 1 – 2$ experiments). In contrast to our

hypothesis, exposure to hyperglycemia did not impact markers of autophagy, and miR-92a manipulation had no further effect.

Conclusions

Our preliminary data demonstrate that while miR-92a impacts production of H_2O_2 to shear stress in health and disease, it does not appear to modulate autophagy responses in endothelial cells or in response to hyperglycemic stress. Future studies could examine whether H_2O_2 production is impacted by hyperglycemic stress and the impact of miR-92a on these responses.

Alisha Hoefs – Baylor University
Peter S. LaViolette, PhD, MS
MCW Department of Radiology

Radio-pathomic maps of tumor pathology identify reduced necrosis in response to bevacizumab

Background

Each year around 15,000 cases of glioblastoma (GBM) are diagnosed. GBMs are the most aggressive form of astrocytoma with only 6.8% of patients surviving five years after diagnosis[1]. Standard treatment of GBM includes surgical resection followed by concomitant chemo-radiation. Bevacizumab is an antiangiogenic agent that is also often used to slow tumor progression and aid in quality of life. This study utilized radio-pathomic maps to identify GBM imaging characteristics outside of enhancement, specifically necrosis and hypercellular tissue, over the course of bevacizumab treatment. It was hypothesized that, as bevacizumab treatment progressed, tumor presence outside of enhancement would be reduced.

Method

This study included 26 GBM patients who had MRI acquired prior to initiation of bevacizumab treatment and additional imaging timepoints throughout treatment. Radio-pathomic models from a prior study were developed using patient MRI scans and patient tissue samples collected at autopsy[2]. Cell density (Cell) and extracellular fluid density (ECF) maps derived from the models were compared to T1-weighted post contrast images (T1C) to distinguish necrotic and hypercellularity outside of contrast enhancement. Both characteristics were manually outlined using ITK-Snap software, and volume and surface area were calculated using MATLAB software. A mixed model linear regression analysis was utilized to compare necrosis and hypercellular tissue volumes and surface areas to bevacizumab duration at each timepoint, accounting for subject as a random effect.

Results

Necrosis volume significantly decreased over the course of bevacizumab treatment ($p = 0.002$), whereas hypercellular tissue volume was not significantly different ($p = 0.357$). Necrosis surface area also significantly decreased over the course of bevacizumab treatment ($p = 0.04$), while hypercellular tissue surface area was not significantly different ($p = 0.841$). Using an estimate of marginal means, necrosis volume and surface area were estimated to reduce by 50.515 mm^3 and 25.929 mm^2 per day of bevacizumab treatment, respectively.

Conclusion

The results indicate that bevacizumab appears to reduce necrotic tissue found outside of contrast but does not appear to have any effect on hypercellular tissue. This implies that GBM patients with a primary necrotic component may have improved survival outcomes with bevacizumab treatment compared to patients with primarily hypercellular tumors. This tool could be useful in identifying patients that would best benefit from bevacizumab treatment and therefore drive clinical decision making.

References

1. Ostrom, Q. T. *et al.* The epidemiology of glioma in adults: a “state of the science” review. *Neuro. Oncol.* **16**, 896–913 (2014).
2. Bobholz, S. A. *et al.* Radio-Pathomic Maps of Cell Density Identify Brain Tumor Invasion beyond Traditional MRI-Defined Margins. *AJNR. Am. J. Neuroradiol.* **43**, 682–688 (2022).

Jordyn Hamburger – Marquette University
Peter S. LaViolette, PhD, MS
MCW Department of Radiology

Glioblastoma necrosis and hypercellularity volumes determining molecular subclasses of tumor

Background

The Cancer Genome Atlas Glioblastoma Multiforme (TCGA-GBM) is a data collection focused on connecting cancer phenotypes to genotypes via clinical images[1]. The phenotype subclasses are proneural, neural, classical, mesenchymal, and glioma CpG island methylator phenotype (G-CIMP), and influence survival outcomes. We hypothesized that the amount of necrosis and hypercellularity observed on radio-pathomic maps can be used to determine phenotype subclasses.

Methods

This study used a previously generated data set of GBM patients with respective molecular subclasses. A previously published model was used to generate radio-pathomic models for cell density (Cell), extracellular fluid density (ECF), and tumor probability maps (TPM)[2]. We annotated hypercellularity and necrosis on radio-pathomic maps, on a set of 98 patients who had presence of both patterns, to determine the relationship between subtype and necrosis or hypercellularity volumes. The initial T1-weighted post contrast (T1C), ECF, and TPM scans for each patient were annotated using ITK-SNAP for presence of hypercellularity and necrosis. Volumetric data calculated across both regions of necrosis and hypercellularity, using MATLAB, included volume, equivalent diameter, convex volume, solidity, and surface area. A linear regression analysis was used to identify differences in the amount and spread of hypercellularity and necrosis based on the designated molecular subtype.

Results

The results initially disagree with the hypothesis that total volume of necrosis and hypercellularity patterns can determine tumor subclass. However, it was found that convex volume of necrosis (trending) and hypercellularity can differentiate between subclass types ($p = 0.027$, $p = 0.064$). Particularly, hypercellularity convex volume was significantly different in the neural and G-CIMP subclass. Neural and G-CIMP medians were above proneural and classical, interquartile range. Surface area of necrosis was also significantly different between subclasses ($p = 0.043$). Surface area of necrosis in the classical subclass was significantly different compared to the other subclasses having the lowest number of interquartile values. Surface area of necrosis was also significantly different between subclasses, with mesenchymal subclass the highest median surface area of necrosis.

Conclusion

Convex volume and surface area reflected the overall spread, which shows greater genetic differences than the total tumor burden. This data pattern on convex volume could be used to determine GBM subclass type without genome sequencing, and eventually determine proper treatment protocol based on respective molecular subclass.

References

1. Beig, N. *et al.* Radiogenomic-Based Survival Risk Stratification of Tumor Habitat on Gd-T1w MRI Is Associated with Biological Processes in Glioblastoma. *Clin. cancer Res. an Off. J. Am. Assoc. Cancer Res.* **26**, 1866–1876 (2020).
2. Bobholz, S. A. *et al.* Radio-Pathomic Maps of Cell Density Identify Brain Tumor Invasion beyond Traditional MRI-Defined Margins. *AJNR. Am. J. Neuroradiol.* **43**, 682–688 (2022).

Margaret Allen – University of Notre Dame
Yang Wang, MD PhD
Department of Radiology, Medical College of Wisconsin

Detecting Cerebrovascular Reactivity Abnormalities in Prodromal Alzheimer's Disease Using Advanced Neuroimaging

Background

Alzheimer's Disease (AD) is an underlying progressive neurodegenerative process which can be characterized along a continuum from prodromal AD to dementia with cognitive and functional disturbances. Prodromal AD, with symptoms of Mild Cognitive Impairment (MCI), is of specific interest as this preclinical form could provide useful biomarkers which can allow earlier diagnosis of AD. MCI can be subdivided into amnestic MCI (aMCI) and nonamnestic MCI (naMCI). aMCI is often viewed as a precursor to AD. Although neurodegenerative elements were popularly studied as a basis for AD, there are notable discoveries which posit that cerebrovascular effects may be causational. In order to investigate these components, cerebrovascular reactivity (CVR) is an important measure which can be used to quantify the cerebrovascular health of the brain.

Method

This study aims to investigate CVR changes in elders diagnosed with MCI, with two groups aMCI and naMCI, in comparison to age-matched healthy controls (AMHC). Through blood-oxygen level dependent functional magnetic resonance imaging (BOLD fMRI), CVR was assessed in 43 human subjects (22 AMHC, 11 aMCI, 10 naMCI). CVR maps were generated from BOLD data. Data was preprocessed and analyzed using AFNI. Group analysis t-tests were conducted between each group. A post hoc comparison was performed on the aMCI and naMCI groups. Age, gender, and education were used as covariates. Regions of interest (ROIs) were identified for further analysis. The analyses were corrected for multiple comparisons using the Bonferroni method. Clinical measures examining executive and memory function were used to assess partial and bivariate correlations between neuropsychological components and CVR trends.

Results

There is a significant association between lower CVR and those with cognitive impairment. CVR was found to be lower in those with MCI compared to AMHC. Those with aMCI were found to have lower CVR in comparison to AMHC and naMCI. Correlations were found within the age covariate as CVR decreased as age increased, however, gender and education did not impact the results. Clinically, there were associations with decreased executive and memory function via neuropsychological testing in relation to lower CVR.

Conclusion

MCI, especially aMCI, may have notable cerebrovascular aspects which can serve as preclinical biomarkers for dementia-based diseases such as AD. There are a variety of neurobiological and cerebrovascular mechanisms which could underlie these changes. The isolated effects of CVR in MCI are informative about the preclinical cerebrovascular state in AD and provide a basis for further research into the vascular hypothesis of AD causation.

Keywords

Alzheimer Disease, Cerebrovascular Circulation, Cerebrovascular Disorders, Cognitive Dysfunction, Magnetic Resonance Imaging, Neurovascular Coupling

A gutsy approach: the long-term effect of B cell depletion MS therapies on the gut

Lauren Simmons[1,2,3], Savannah Neu[2,3], Bonnie Dittel[2,3]

Duke University[1]; Department of Microbiology & Immunology, Medical College of Wisconsin[2]; Blood Research Institute, Versiti[3]

Multiple sclerosis (MS) is an autoimmune, neurodegenerative disease of the central nervous system (CNS) that afflicts both the patient's physical and mental well-being. While there is currently no known cure for MS, αCD20 B cell depletion therapies have shown promising results in clinical trials, such as elongation of relapse rate and reduction of disease progression. These therapies use monoclonal antibodies that target the pan B-cell surface marker CD20. While each available therapy seems to have a similar effect on disease severity in patients, the mechanisms of αCD20 depletion are not well understood. Equally poorly-understood is whether the duration of therapy use has an effect on disease mitigation and overall body system health. To better understand the consequences of long-term αCD20 use, this study examined B cell depletion in the gut after continuous dosing with αCD20 for several months, using IgA as a proxy for B cell presence and bacteria's binding ability in the gut. IgA is an antibody that is abundant in the gut and mediates homeostasis between the gut microbiome and the host organism by binding to bacteria. Human CD20 transgenic (hCD20tg) mice and littermate controls were treated with either rituximab, a weakly depleting αCD20, or 2H7, a strongly depleting αCD20. After either short-term depletion (2 doses over the course of three weeks) or long-term depletion (5 doses over the course of 5 months), various data were collected including 1) quantification of immune cell subsets in several tissues by flow cytometry, and 2) analysis of fecal matter for IgA via BUG-flow. Flow cytometry showed that both of the αCD20 antibodies significantly decreased B cells in the gut. However, BUG-flow showed that αCD20 therapy had a negligible effect on levels of IgA-bound bacteria. These studies have shown that αCD20 therapies appear safe for long-term use in general, but whether these therapies give rise to detrimental side effects downstream will need further study. Future experiments should investigate if there are any side effects of excessive B cell depletion, especially within the CNS, and how they may impact MS models such as experimental encephalomyelitis (EAE). These efforts will ultimately contribute to identifying the most effective future treatments for MS.

Maryam Aftab – Mount Mary University
Dr. Karin Hoffmeister, MD
Versiti Blood Research Institute, Medical College of Wisconsin

---

Cultivating Cell Culture Lines on Phase I of XmAB18968 (CD3-C38) for Treatment of Patients Inhibited with Relapsed/Refractory Acute Leukemia and T-cell Lymphoblastic Lymphoma: Observing the Growth of OCI-AML3 and MV4-11

Background

Acute myeloid leukemia is a type of cancer that starts to develop into varying types of blood cells. With acute myeloid leukemia (AML), the condition starts within the soft inner part of certain bones or the bone marrow where new blood cells are often made. This uncontrolled and erratic growth many times spreads to other portions of the body such as the central nervous system, liver, spleen, and lymph nodes. CD38 is a type of glycoprotein that specializes in signal transduction, migration, as well as the process of cell adhesion. It is often used as a marker for chronic lymphocytic leukemia prognoses. As a type II transmembrane glycoprotein, CD38 antibodies have been developed in use for immunotherapies due to their antibody effector functions which are induced through the Fc region of the selected antibody - further including complement dependent cytotoxicity, antibody-dependent cellular phagocytosis, and antibody-dependent cellular cytotoxicity. The antibodies of the transmembrane glycoprotein CD38 have the ability to ameliorate immunity of host-anti tumors through the process of obliterating regulatory B and T cells as well as suppressor cells that are myeloid related and driven. In order to profile and derive the role and mechanisms of CD38 glycans in regard to immune response, the cell culture lines OCI-AML3 (ACC 582) and MV4-11 (ACC 102) containing acute myeloid leukemia were retrieved, observed, and properly maintained within their perspective cell culture mediums. Maintaining and growing cell cultures is paramount and regarded as one of the most important techniques within the scope of cellular biology as it serves as a platform to investigate the physiology, biology, and the metabolism of diseased cells such as the acute myeloid leukemia (AML) cell lines observed. The scope of this experiment aims to identify the role that the glycan CD38 exhibits - specifically in its expression in AML cells that are therapy-resistant, patterns of composition when dealing with malignant AML cell lines, as well as the role of expression in XmAb18968 target recognition and certain effector functions.

Method

Observations were made in regard to cell line samples containing acute myeloid leukemia, OCI-AML3 (ACC 582) and MV4-11 (ACC 102), which were retrieved and received from two male humans. The cell culture medium was maintained at 80-90% RPMI 1640 + 10-20% h.i FBS. OCI-AML3 (ACC 582) was preserved and maintained at 0.4-1.0x10^6 cells/ml whilst MV4-11 (ACC 102) was maintained at 0.5-2.0x10^6 cells/ml. Both cell cultures were incubated with 5% carbon dioxide levels at 37°C. These are the proposed ideal levels and criterion at which both OCI-AML3 (ACC 582) and MV4-11 (AC 102) are believed to grow in optimally.

Results

Under these conditions the cell culture lines within their perspective mediums grew optimally with minimal deterrence and high viabilities. Cultures presented to be healthy with viabilities >90% with no apparent signs of microbial contamination.

Conclusion

These cells will be stored and further utilized through the progression of the study at the Hoffmeister lab and allow for more direct identification of the CD38 glycoprotein, the role of glycosylation, and how resistance patterns are presented within acute myeloid leukemia patients undergoing anti-CD38 therapies. The experiment aims to more deeply understand the role of the expression of CD38 in malignant cancers.

Madeline Olson – University of Wisconsin – Stout
Department of Cell Biology, Neurobiology, and Anatomy, MCW
Allison Ebert, PhD

The Role HCMV Plays in Alzheimer's Disease

Background

Human cytomegalovirus (HCMV) is a common herpesvirus infection that affects 40-70% of the population. Other studies estimate that underdeveloped nations have even higher rates of infection. HCMV has been linked phenotypes common in neurodegenerative diseases, such as the increased amounts of Amyloid beta (Aß) and phosphorylated Tau (pTau) found in Alzheimer's Disease (AD). AD is the most common neurodegenerative disease affecting over 6 million people in the US. Here, we will examine the role that HCMV plays in Aß and pTau accumulation using an induced pluripotent stem cell (iPSC)-derived forebrain neuron culture system and the clinical HCMV variant TB40/E-eGFP. We hypothesize that HCMV increases both pTau and Aß deposits in neurons, a phenotype with implications relevant to AD pathogenesis. We intend to test this theory using a series of assays, including western blots and enzyme-linked immunosorbent assays (ELISAs). Preliminary results indicate that HCMV increases levels of pTau in neurons generated from both control and AD individuals. Interestingly, early results show that secreted amounts of toxic Aß seem to decrease with HCMV infection. Taken together, these results highlight a complex relationship between HCMV and AD pathology.

Method

The purpose of this study is to explore the relationship between HCMV and Alzheimer's disease pathology, specifically the increase of Alzheimer associated proteins in infected neurons. To demonstrate this potential relationship, a western blot was conducted to investigate the protein expression found in healthy control and AD neurons without and with HCMV infection. An ELISA was also administered to study the release of the toxic Aβ protein.

Results

The results from the ELISA showed a decrease in the secretion of the toxic Aß protein whereas that western blot analysis showed an increase in the accumulation of insoluble pTau protein.

Conclusion

Alzheimer's disease pathology is complex, and the preliminary results from this study suggest that HCMV infection affects Alzheimer's disease-like pathology in human neurons. HCMV infection clearly shows an increase in insoluble pTau protein accumulation in both control and AD iPSC neurons. However, soluble Aβ appears to be reduced with HCMV infection. These data suggest that pTau and Aβ have different mechanistic responses to HCMV infection that require additional research. Future experiments will test if the reduction in secreted Aβ is due to innate immune activity or a transition to a more insoluble form similar to what is observed for pTau. This study is important because of the commonality of both Alzheimer's and HCMV infection, so understanding their relationship is valuable for future scientific exploration.

Keywords: Alzheimer's, Insoluble Protein, Induced Pluripotent Stem Cells, Phosphorylated Tau, and Amyloid Beta

Emma Tabor– Marquette University Antje
Kroner-Milsch, MD/PhD
VA Department of Neurosurgery

HEME BINDING PROTEINS MINIMIZE SECONDARY DAMAGE IN SPINAL CORD INJURIES

Background

Spinal cord injury (SCI) is a severe condition resulting in significant neurological deficits. In an SCI, tissue damage occurs in two major phases: The primary damage is caused by the initial mechanical trauma. This is followed by secondary damage, which is caused by various factors, including inflammation and hemorrhage. This project aims to improve SCI outcomes by minimizing the factors that cause secondary damage to the tissue.

When an injury is sustained and a hemorrhage occurs, red blood cells lyse in the tissue, thereby releasing lysis products including hemoglobin and its derivatives such as heme. These products are toxic and react with proteins and lipids damaging nearby structures. Alpha-1 microglobulin (A1M) and alpha-1 anti- trypsin (A1AT) are binding proteins that provide a natural detoxification of these blood breakdown products. A1M and A1AT could provide support in eliminating heme toxicity in the tissue.

In this project we are investigating the role of A1M and A1AT as heme binders to minimize secondary tissue damage and promote better recovery following an SCI.

Methods

First, we characterized the presence of A1M and A1AT after SCI using a mouse model of contusion SCI. We performed enzyme-linked immunosorbent assays (ELISA) to quantify the amount of protein found at the lesion site after a SCI. Secondly, we assessed how different concentrations of A1M and A1AT treatment protect cells from damage induced by hemoglobin products. We evaluated cellular damage using a reactive oxygen species assay. The third objective is to evaluate A1M and A1AT as a therapeutic treatment for SCI. In ongoing experiments, A1M and A1AT will be injected into the perspective lesion site in mice, and their behavior will be analyzed to assess functional improvements.

Results

We confirmed that the heme binding proteins are upregulated in the spinal cord as a result of injury. Analysis of ELISA test for A1AT show that this protein is upregulated after an SCI compared to an uninjured control group around day 1-3.

Using a culture of bone marrow derived macrophages, we have shown that increasing concentration of A1AT reduces the amount of damaging reactive oxygen species in the presence of hemin. For example, treatment with 150um of A1AT decreased ROS production by more than 50%.

Conclusion

A1AT and A1M are upregulated in the spinal cord following an SCI. Increasing concentrations of these proteins have the potential to minimize the secondary damage at the lesion site caused by inflammation and hemorrhage.

Shariq Zaman – Illinois State University
Antje Kroner-Milsch, MD, PhD
MCW Department of Neurosurgery
Zablocki VA Medical Center

ROLE OF THE CCR5 PATHWAY IN TISSUE PROTECTION AND RECOVERY AFTER SCI

Background

Spinal cord injury (SCI) results in severe neurological impairment, depending on size and localization of the tissue damage. While primary damage is caused by the mechanical trauma from the initial injury, secondary damage is caused by a series of events that occur after the injury, including exacerbated inflammation with hemorrhage, apoptosis, and oxidative stress. This increases the lesion size and affects functional recovery. To reduce tissue damage and improve functional outcomes after SCI, there is a need to investigate the mechanisms by which secondary damage takes place. C-C chemokine receptor 5 (CCR5) and its ligand macrophage inflammatory protein 1-alpha (CCL3) are pro-inflammatory and have been implicated as detrimental factors after SCI. However, their impact on neuronal plasticity is not well understood. In other neurotraumatic conditions, inhibition of the CCR5 pathway resulted in improved recovery and was associated with upregulation of transcription factor cAMP-response-element-binding protein (CREB) signaling. In this study, we are examining downstream effectors of the CCR5 pathway and the effects of CCR5 inhibition on recovery after SCI.

Method

In an ongoing experiment, we are using a mouse model of lower thoracic (T11) spinal cord contusion injury. CCR5 inhibition is achieved by administration of an FDA-approved CCR5 antagonist (maraviroc) at a concentration of 20 mg/kg intraperitoneally for a duration of 7 days. Functional outcome of injured mice is assessed using behavior testing for locomotor recovery. Liver function will be examined at the experimental endpoint to identify hepatotoxicity as a potential side effect of maraviroc.
In addition, immunofluorescent staining of spinal cord cross sections was performed to determine which cell types express phosphorylated CREB (pCREB). Expression levels will be compared between tissue obtained from injured and uninjured wild-type and $CCL3^{-/-}$ mice to determine whether the absence of CCL3 leads to increased pCREB signaling and mild improvement in tissue protection and recovery after SCI.

Results

We have preliminary data suggesting that maraviroc treatment improves locomotor recovery and tissue preservation after SCI.
Staining of spinal cord cross sections indicated that pCREB is predominantly expressed in neurons, but it is also found in ependymal cells as well as in some microglia and oligodendrocytes. We have not observed pCREB expression in astrocytes.

Conclusion

Knockout of CCL3 and treatment with a CCR5 antagonist are methods that both partially or fully inhibit CCR5 signaling. Our findings indicate that CCR5 may be a useful therapeutic target to control the inflammatory response and reduce functional impairment caused by secondary damage after SCI.

Keywords

CCR5; CCL3; pCREB; Inflammation; Secondary Damage; Spinal Cord Injury

Faeq Zaman – Illinois State University
Elizabeth Sweeny, PhD
MCW Department of Biochemistry
Medical College of Wisconsin

NOX5 ACTIVATION MODIFIES THE PROTEASOME AND AFFECTS ITS ACTIVITY

Background

NADPH Oxidase 5 (NOX5) is a transmembrane signaling enzyme that produces superoxide, a reactive oxygen species (ROS) formed in response to calcium flux. Superoxide is formed by the addition of an extra electron to molecular oxygen through NADPH electron exchange. Involved in oxidative signaling, ROS are necessary for healthy cell function; however, elevated levels of ROS are associated with cell damage and the initiation of disease. During mild oxidative stress, the proteasome assists in maintaining homeostasis and modulating the effects of ROS by being the primary pathway for regulating protein concentrations and degrading damaged proteins. Insufficient degradation, high ROS levels, and increased redox cycling causes an accumulation of oxidized proteins and their aggregates. These aggregates can then inhibit the proteasome by oxidizing its subunits. Our current knowledge on how the proteasome is regulated under oxidative stress is quite limited. Because ROS levels are influenced by several factors, understanding how one producer of ROS affects the proteasome can assist in developing strategies to cope with oxidative stress and prevent the development of diseases. A mass spectrometry screen we performed identified many of the proteasome subunits being oxidatively modified by NOX5-induced ROS. Therefore, here we established how NOX5 activation modifies the proteasome and affects proteolysis.

Method

HEK293 cells were transfected with NOX5 or an empty vector control and then treated with DMSO alone or with ionomycin to induce a NOX5 superoxide burst. To assess the effect of NOX5 activation on proteasome function, we used western blot analysis of ubiquitinated proteins and substrate degradation, a florescent proteasome activity-based probe, and degradation of a fluorescently labeled proteasome substrate. Finally, pull-down assays probing for free cysteine residues were performed to confirm which proteasome subunits NOX5 was modifying.

Results

Our preliminary data suggests that NOX5 activation causes an inhibitory modification of the proteasome. Decreased proteolysis was observed in cells with activated NOX5 by a build-up of ubiquitinated proteins, a decreased fluorescence signal of our activity-based probe, and an increased fluorescence signal of our substrate probe.

Conclusion

Proteasome inhibitors play a key role in the treatment of diseases such as multiple myeloma. However, in some patients, these drugs have been noted to cause increased susceptibility to adverse cardiac events. By establishing the impact of NOX5-induced ROS on the proteasome, a stronger control over proteasome activity can be developed to help prevent these adverse reactions.

Keywords

NOX5; Proteasome; Protein Degradation; Reactive Oxygen Species; Oxidative Modification

Kathryn Bingenheimer - Purdue University
Dawn Wenzel, PhD
Department of Biochemistry, Medical College of Wisconsin

Exploring Interactions in Autophagy: Mapping the ULK3 Binding Site with ATG13

Background

Autophagy is a regulated process in which cellular components are targeted for degradation in the necessary maintenance of cellular homeostasis. Autophagosome initiation is driven by a single kinase called Atg1 in yeast, while humans have three kinases known as ULK1-3 that can promote autophagosome biogenesis. ULK3 regulates the cell cycle checkpoint known as the abscission checkpoint and promotes autophagy of the post-mitotic midbody remnant, a structure that has important roles in development and differentiation. How the molecular machinery that drives midbody remnant autophagy compares with canonical ULK1/2-mediated autophagy and how ULK3 function in autophagy is coordinated with regulating the abscission checkpoint is completely unknown. ULK3 binds ATG13 via two C-terminal Microtubule Interacting and Transport (MIT) domains to form a complex that is required for autophagosome formation during autophagy. ULK3 also uses MIT domains for protein-protein interactions required for the regulation of abscission. My goal is to further map the ULK3 interacting residues on ATG13 and those within the ULK3 MIT domains to determine if ULK3 binds a unique region on ATG13 (compared to ULK1/2) and whether ATG13 binding competes with other abscission checkpoint binding partners that bind ULK3 MIT domains.

Method

I used a two-fold approach to map out the interactions between ULK3 and ATG13. The first method involved C-terminally truncating ATG13 to identify the point at which binding between ATG13 and ULK3 ceased. The ATG13 truncations were transfected with ULK3 and then purified in a pulldown assay which also tested for binding between the constructs, visualized via Western blot. The second approach was to create a series of point-mutations in the MIT domains of ULK3 and test for binding with ATG13. Constructs were created via Gibson cloning and then used in co-transfection experiments similar to those performed with ATG13 truncations and were also used to test for ULK3-binding.

Results

I successfully generated 5 point-mutations in the MIT domains of ULK3. These point mutations test whether ULK3 binds ATG13 via analogous MIT surfaces as those predicted for ULK1/2 kinases and whether ATG13 competes with MIT domain binding with the abscission checkpoint protein IST1. Co-transfection experiments with these MIT domain contracts and the ATG13 truncations are ongoing.

Conclusion

Mapping the interactions between ULK3 and ATG13 aid in the discovery of the structure-function relationship between the two constructs. My research study will provide a foundation for exploring the role ULK3 plays in autophagy regulation.

Iman Manzoor – Wayne State University

Christopher M. Olsen, PhD

MCW Department of Pharmacology and Toxicology

Whole Brain Analysis of Ensemble Activation in Mice via iDISCO

Memories are believed to be encoded in neuronal networks called ensembles. These neurons fire in a coordinated way to encode a specific memory and are crucial for learning. Ensemble reactivation in drug seeking behavior is being studied in rodents by the Olsen lab. The typical method for studying ensembles is to use c-Fos immunohistochemistry to stain for active neurons in microscopically thin (30um) brain sections, made by using a cryostat. However, the cryosectioning process is slow, tedious, and limits the volume of tissue that can be imaged. This project attempts to visualize neuronal ensembles in larger sections (1-3mm) of the brain, using the immunolabeling-enabled three-dimensional imaging of solvent-cleared organs (iDISCO) protocol created by Reiner et al. Mice underwent a two-part tail-restraint experiment**,** then were perfused and the extracted brains were sliced manually into 1-, 2-, and 3-mm sections. The iDISCO protocol was followed – samples were first pre-treated with methanol which included dehydration with a methanol/H2O series, delipidation with dichloromethane, and decolorization using hydrogen peroxide. Brains were immunolabeled for c-Fos then further cleared. Samples were incubated in dibenzyl ether (DBE) for refractive index matching before being imaged using confocal microscopy. The goal was to be able to clear the brain sections well and to be able to visualize the neuronal ensembles that were involved in the tail-restraint test.

Sophia Aliaga – University of Wisconsin-Milwaukee
Christopher Olsen, PhD
Department of Pharmacology and Toxicology

Whole Brain Identification of Neurons Projecting to the Cervical Spinal Cord

Background

This research is a collaboration with Kajana Satkunendrarajah's laboratory. The laboratory injected AAVrg-CAG-tdTomato virus into the cervical spinal cord, which is an ideal fluorescent protein for live animal imaging studies. 20-micron coronal sections were taken from the mouse brain resulting in 250 brain sections. This research integrated three-dimensional imaging in order to identify tdTomato cell bodies. Understanding how to use three-dimensional imaging provides the opportunity to discover scientific and medical findings due to the large visualization scale on which the brain can be examined. Specifically, the software: Imaris, FileBuilder, QuickNII, VisuAlign, and Nutil were put into practice to develop a further understanding of, first, identifying location of neurons that send axonal projections to the cervical spinal cord, and the quantity within each brain region and, second, how three-dimensional imaging can provide new information in the research and medical world.

Method

Imaris software was used to transform the IMS files supplied by the imaging facility onto TIF files. From there, the study used ImageJ (FIJI) software to develop lower-resolution files, either JPEG or PNG. FileBuilder was then used to create an XML segmentation, which created a network for all the images. The study created three XML segmentations folders and used NOTEPAD to combine the coding into one document. QUICKNII was used to cut planes through the atlas templates that would match the orientation of the cutting plane of the 2-dimensional experiment images. VisuAlign was then used for nonlinear refinements to the existing 2D-3D registration created on QUICKNII. IMARIS was the next step to generate spots from the tdTomato to find the cell bodies. Nutil Quantifier was used to quantify the number of cell bodies in each brain region to then develop results. Protocols were also developed for all the software mentioned for future usage of the Dr. Olsen Laboratory.

Results

When results are collected, we will be able to identify the location and quantity of neurons sending axonal projection to the cervical spinal cord.

Conclusion

Three-dimensional imaging allowed for a precise identification of neurons that were sending axonal projection to the cervical spinal cord using the images from the Kajana Satkunendrarajah's laboratory. This research also allowed for a further understanding of how three-dimensional volume imaging can be the next tool used when wanting to develop brain images.

Victoria Toledo – University of Notre Dame
Aasim Padela, MD, MSc, FACEP
MCW Department of Emergency Medicine

Questions at the Intersection of Bioscience and Religion

Background
In the past few centuries, science and religion have appeared to become increasingly oppositional. In the past, however, the two have shared a symbiotic relationship, with the natural sciences and philosophy driving the study of the other. In an increasingly polarized world, it is urgent that these divisions be addressed in order to build social and civic bridges. To address the shared questions of bioscience and religion, a narrative review was conducted on scientific and theological perspectives as they relate to human origin, essence, determinism, and uniqueness.

Method
Print and digital sources available from the MCW Library, Scopus, PubMed, Web of Science, and scholarly contacts were reviewed. While searching, a specific focus was kept on biology and the three Abrahamic religions (Judaism, Christianity, and Islam). A record was kept of themes and ideas addressed by each relevant source, and these were compiled into various summary documents related to each question.

Results
An extensive narrative review showed that these questions were discussed and even debated within each scholarly field; certain dimensions of each issue differ across each major religious tradition and within religious denominations. The scientific perspectives tended to lean towards simple materialism. Despite these differences, however, there appeared to be a common consensus that neither science nor religion had full explanatory authority on any issue. One example is the question of sentience and how it can be explained by both neural networks and the supervenience of a soul.

Conclusion
The literature review indicates that there are, in fact, shared questions between bioscience and faith which continue to be discussed today. It was found that between science and religion there were many instances of overlap between their issues, indicating that science and religion, in their middle ground forms, are not oppositional but rather complimentary. A greater understanding of the continued symbiotic relationship between science and religion can help to address issues of scientific rejection, such as vaccine hesitancy, and open discussions about interfaith collaboration and the importance of religious diversity in academic and professional settings. This knowledge can be used towards a medical school elective which recognizes the religious identities of students and helps them to build their interreligious competency for patient care settings.

Afiya Quryshi – Harvard University
Aasim Padela, MD, MSc, FACEP
MCW Department of Emergency Medicine

Investigating American Muslim Palliative and Hospice Care Needs

Background

Muslims comprise over a quarter of the global population, and diasporic communities continue to grow. In the United States, almost 65% of Muslims are immigrants and 14% are over the age of 55. With both numbers being on the rise, there is an increased need for palliative and hospice care. However, research shows that Muslims under use palliative and hospice care services, one reason being Muslims' healthcare seeking behaviors are largely guided by religious values. Moreover, there is no Muslim-run hospice program in the U.S, furthering the gap between the Muslim community receiving adequate support through end-of-life care. Thus, the unmet needs and challenges faced in end-of-life care by the Muslim population must be identified and categorized.

Method

To identify the unmet needs and challenges faced in end-of-life care by the Muslim population, a systematic literature review is being conducted. First, records were identified through five databases: Ovid MEDLINE, Scopus, Web of Science, CINAHL, and Cochrane Library. Records were identified using search terms such as "Islam," "Muslim," "palliative care," "assisted death," and "living will". All articles were imported into Rayyan, a systematic literature review organizer. Then, 1004 articles were screened based on inclusion criteria: empirical studies, 18+ population studied, studies done in a diasporic context (high-income, minority Muslim countries), and studies written in English.

Results

After screening in Rayyan, 59 papers remained included, 67 papers are labeled as "maybes" and are in need of further full-text review, and 873 are excluded. Next, through the process of data abstraction, we are compiling the research design, population studied, care type studied, and which of the 8 domains of palliative care (structure, physical, psychological, social, spiritual, cultural, nearing end-of-life, legal) studied, for each of the included papers.

Conclusion

The outcome of this project is to identify the areas that Muslim patients and caregivers struggle with in palliative, hospice, and end-of-life care in general. By engaging with both literature and the community itself, we will be able to tailor services specifically to peoples' unmet needs. Specifying the challenges that the Muslim community faces will help create interventions for providers to develop more culturally relevant and religiously sensitive models for care delivery. Creating a Muslim specific hospice program in the U.S. will begin to bridge the gap between the Muslim community and accessing the end-of-life care they need.

Reethika Veluri – Saint Louis University
Raul Urrutia, MD; Michael T. Zimmermann, PhD
MCW Mellowes Center for Genomic Sciences and Precision Medicine

---

Computational Studies on 85 Novel Mini Proteins Lend Insight into Their Structure-Function Relationship

Background

Mini proteins (functional polypeptides containing <50 AA) have recently elicited significant interest due to their role in fundamental functions for biological processes and diseases. These proteins are also of importance in biotechnology and drug development. Notably, the recent completion of the human genome has provided information on the existence of a large amount on novel mini proteins, which properties remain to be defined. Thus, in this study, we determine a process of how to analyze and score previously uncharacterized mini proteins using classic sequence-based bioinformatics, along with computational biophysics and enhanced extensive system biology annotation to test the following hypothesis: these approaches will provide novel information that describe the structure-function relationship of a subset of mini proteins with potential biomedical relevance.

Method

We analyzed a group of 85 eukaryotic mini proteins using a multi-tier approach. First, we performed sequence-based analysis of these proteins using several algorithms that can identify regions of structural and functional importance. Subsequently, we used structure-based scores based on the development and 3D models using two different AI-based methods. Structural analysis of these models was performed using several computational biophysics methods to obtain meta-models that could inform on the evolution, structure, and likely functions of these proteins.

Results

We find several relations between predicted structure and functional properties to assign these proteins to several groups with similar properties. Sequence-based analysis leads us to identify motifs and residues that link structure-to-function for most of these proteins. Structure-based scoring led us to build 3D models which provided significant complementary information.

Conclusion

Combined, both of these steps have allowed us to categorize mini proteins based not only on sequence, but also according to their structure and potential function. Thus, the identification and novel properties for the mini proteins reported here, bear significant mechanistic and biomedical relevance.

Trinity Higgins – Western Carolina University
Kirsten M. M. Beyer, PhD, MPH, MS
MCW Institute for Health and Equity

Racial links to disparities in breast cancer treatment

Background
Breast Cancer Race and Place (BCRP) is a study on structural racism and breast cancer survival disparities in Milwaukee, Wisconsin. There are three main aims to this study, however, only aim three will be addressed in this project. Aim three explores how Black, Hispanic and non-Hispanic White breast cancer survivors residing in a highly segregated area (Milwaukee, WI) have been impacted during cancer treatment in the context of race and segregation.

Method
Qualitative narrative interviewing and analysis were used to understand the health care experience of breast cancer survivors in Milwaukee, WI. These interviews are primarily done via telephone. At the beginning of the interview, participant demographics are collected, including residential history. The interview is semi-structured using a life history calendar approach. The participant is asked to describe her experience with breast cancer, from diagnosis to the present. These interviews are then transcribed and coded using a software called MAXQDA. Interviews are coded using a codebook established by the project team based on a conceptual model and free coding of the initial set of participant interviews to maintain consistency. The coded interviews are then subject to analysis to identify themes and compare themes across racial and ethnic groups. Ten interviews from each of the three racial/ethnic groups (Black, Hispanic and non-Hispanic White) cancer survivors were used for this comparative analysis.

Results
Several key, reoccurring themes presented themselves and a comparative analysis revealed clear differences between the minoritized survivors and the White survivors. Major themes that surfaced in the Black and Hispanic interviews included transportation barriers, lack of economic support, cultural/language barriers, and poor treatment by the healthcare team. These themes appeared much less frequently, if at all, in the experiences of the White cancer survivors. Using codes from MAXQDA, it was found that codes describing racism and discrimination were found in greater number within the Black and Hispanic groups versus the non-Hispanic White group.

Conclusion
This comparative, qualitative study identified different breast cancer survivorship experiences based on patient race and ethnicity in the Milwaukee metropolitan area. The findings of this study can be used to help advocate for better practices in medicine, as well as policy changes to help reduce disparities.

Deja Kabore– University of Wisconsin, Milwaukee
Jennifer A. Campbell, Abigail Thorgerson, Aprill Z. Dawson, Sanjay Bhandari, Leonarde Egede, MD, MS
MCW Center for Advancing Population Sciences, Health Equity Research Group, MCW SPUR

Dose Response Relationship Between Food Insecurity and Quality of Life in US Adults

Background
Declined general health has been associated with food insecurity, however little is understood about the relationship between food insecurity and other patient reported outcomes, specifically, quality of life. The purpose of this study was to examine the dose response relationship between food insecurity and quality of life in US adults. Using national data collected through the Medical Expenditure Panel Survey (MEPS) 2016-2017 representative of 275,829,365 US adults, the relationship between categories of food security and both physical component score (PCS) and mental component score (MCS) related quality of life were analyzed using linear regression models. The purpose of this study was to examine the dose response relationship between food insecurity and quality of life in US adults.

Method
Data from MEPS 2016-2017 representing US adults aged 18 and older was used. The independent variables: Four categories of food security. Categories included: food secure, marginal food security, low food security, and very low food security. Outcome variable: quality of life was the outcome variable of interest and was measured continuously by the PCS and the MCS. Lower scores represent lower quality of life. MCS and PCS linear regression models were run, both adjusted and unadjusted to account for the following covariates: age, sex, race/ethnicity, education status, employment status, region, poverty status and insurance status.

Results
Results show that with an n=275,829,365 US adults, 6.6% reported marginal food security, 4.9% reported low food security, and 4.6% reported very low food security, adjusting for covariates including age, sex, race/ethnicity, education level, employment status, US region, poverty status, insurance type, and comorbidity count, the relationship showed dose response with marginal food security associated with 1.6 lower points in PCS ($p<0.001$), low food security associated with 2.8 lower points in PCS ($p<0.001$), and very low food security was associated with 3.6 lower points in PCS (<0.001), compared to food secure adults. For MCS, marginal food security was associated with 2.9 lower points ($p<0.001$), low food security was associated with 3.9 lower points ($p<0.001$) and very low food security was associated with 7.9 lower points ($p<0.001$) compared to food secure adults. When dichotomizing the variable to match current standards for food insecurity status and adjusting for all self-reported comorbidities in the dataset, food insecurity was associated with 2.2 lower points in PCS ($p<0.001$) and 5.6 lower points in MCS compared to food secure adults.

Conclusion

Increased food insecurity was associated with decreased physical and mental component quality of life scores. Adjustments for covariates showed statistical significance. This relationship was not explained by factors including demographic factors, socioeconomic factors, comorbidity burdens, or insurance. This study suggests work is needed to mitigate the impact of social risk factors, namely food insecurity, on quality of life due to the association of decreased quality of life with poor healthcare outcomes and increased mortality.

Sex Differences in the Relationship between Social Determinants of Health and Emergency Department Visits in Adults with Diabetes Mellitus

Olaitan Akinboboye, Leonarde E. Egede, Rohan Anne, AT, Joni S. Williams

Abstract:

Background: Diabetes has been associated with increased health and financial burden in the United States. This study examined sex differences in the relationship between social determinants of health (SDOH) and emergency department (ED) visits in adults with diabetes mellitus (DM).

Methods: Data were analyzed on 8,958 U.S. adults in the 2016-2018 National Health Interview Survey (NHIS). The independent variables included 6 SDOH: economic instability, lack of community, educational deficit, food insecurity, social isolation, and inadequate access to care. The primary dependent variable was the number of visits to the ED in the past year. Multiple logistic regression assessed the independent relationship between SDOHs (social determinants of health) (social determinants of health) and ED visits stratified by sex, controlling for covariates such as age, race/ethnicity, insurance status, obesity, mental health status, and co-morbidities.

Results: In the fully adjusted model, social isolation [OR = 1.60 (95% CI: 1.33, 1.93)] and food insecurity [OR = 1.47 (95% CI: 1.19, 1.81)] were statistically associated with ED visits among men. Similarly, social isolation [OR = 1.40 (95% CI: 1.15, 1.70)] and food insecurity [OR = 1.53 (95% CI: 1.26, 1.86)] were significantly associated with ED visits among women. In addition, there was a statistically significant relationship between economic instability [OR = 1.60 (95% CI: 1.33, 1.93)] and ED visits among women, but not among men.

Conclusion: Food insecurity and social isolation are important drivers of ED visits among men and women with DM. In addition to these social risks, economic instability was associated with ED visits among women but not men. Future studies should elicit the mechanism of these relationships.

Nabeel Bhimani – Illinois Wesleyan University
Sara Kohlbeck, MPH
MCW Division of Suicide Prevention

Prevalence of Depression and/or Anxiety among Suicide Decedents in Wisconsin.

Background
Studies have demonstrated a link between depression and/or anxiety and suicide. These diagnoses are common, yet little is known how these diagnoses affect demographic groups among suicide decedents, especially when observing suicides in relation to depression and anxiety in a affective neuroscience perspective. The purpose of this study is to examine the demographic information on Wisconsin suicide decedents who had a diagnosis of depression and/or anxiety at the time of their suicide.

Method
This study uses data from the National Violent Death Reporting System. We look at all the suicides that have occurred in Wisconsin from 2004 to 2018 and were taken from the Wisconsin Violent Death Reporting System. The data was organized by demographics (Sex, Race, Age) to observe any trends in proportions of depression and/or anxiety diagnosis throughout the study period.

Results
The data showed certain trends among each demographic that had a diagnosis of depression and/or anxiety: men (68.35%) had a higher suicide rate than women (31.65%) which seems to show that men who have depression or anxiety are more likely to die by suicide, the sample size for people of color was very small in comparison to those who were white, and those ages 44-60 (39.46%) had a higher suicide rate than all other ages. The sample of those diagnosed with depression and/or anxiety who took their own life was 4417.

Conclusion
The results did show that [39%] of Wisconsinites who died by suicide had either depression and/or anxiety at the time of their suicide. The study shows males have a higher suicide rate and continues to increase as stigmatism of emotions may continue to be prominent in society. Another observation is of how the diagnosis of affective disorders in people of color who die by suicide is small in comparison to those who are white showing possible bias or stigma of mental health issues, or underdiagnosis of these issues, in non-white communities. These results should get an in-depth analysis on how we could use affective neuroscience to understand these ideas better. Literature seems to have a divide in public health and affective neuroscience when the two concepts can be used together to understand how mental diagnoses affect lives which could be helpful in preventing suicide.

Keywords: Affective Neuroscience, Death, Stigma, Literature.

Abigail Genal – St. Norbert College
Christopher Kristich, PhD
MCW Department of Microbiology and Immunology

Analysis of the role of conserved residues on GpsB function in cephalosporin resistance in *Enterococcus faecalis*

Background

Enterococci are Gram-positive, opportunistic pathogens that are a major cause of hospital-acquired infections. Enterococci are intrinsically resistant to cephalosporin antibiotics, making infections caused by these bacteria difficult to treat. GpsB is a cytosolic protein that is required for cephalosporin resistance in *E. faecalis*, but our understanding of the role of GpsB in the mechanism of enterococcal cephalosporin resistance is incomplete. Studies performed with GpsB homologs from other bacterial species indicate that GpsB self-associates and forms hexamers. The structure of GpsB homologs has been solved and reveal that GpsB is made up of two domains, termed the amino-terminus domain and the carboxy-terminus domain. Both domains are thought to contribute to the oligomerization of GpsB, with the amino-terminus domains forming dimers and the carboxy-terminus domains forming trimers. Previous research from the literature identified two conserved residues in the amino-terminus domain and two conserved residues in the carboxy-terminus domain that are important for the oligomerization and function of GpsB homologs. However, the functional importance of the conserved GpsB residues has not been previously investigated in *E. faecalis*.

Method

We hypothesized that the conserved residues are functionally important for the role of GpsB in cephalosporin resistance of *E. faecalis* and for the ability of enterococcal GpsB to oligomerize. To test this hypothesis, we sought to construct four GpsB mutants each with one of the conserved residues mutated to alanine and perform antibiotic susceptibility assays and bacterial adenylate cyclase two-hybrid (BACTH) assays.

Results

Our results suggest that the conserved residues are important for the role of GpsB in cephalosporin resistance but are not each individually responsible for the self-interaction of GpsB.

Conclusion

The conserved GpsB residues affect cephalosporin resistance in *E. faecalis*. They also allow the oligomerization of GpsB to persist. Further investigation is required confirm that the expression and abundance of the GpsB mutants is similar to that of wild-type GpsB in *E. faecalis*, but our results can be used for future studies to further decipher the role of GpsB in the mechanism of enterococcal cephalosporin resistance.

Rhea Singh – Boston University Dr.
Kajana Satkunendrarajah, PhD MCW
Department of Neurosurgery

Cervical Excitatory Interneuron Mediated Hypercapnic Ventilatory Response in Health and Spinal Cord Injury

Background:
Cervical excitatory interneurons (cEINs) receive descending input from the brainstem and form synapses on phrenic motor neurons (PMNs), which then innervate the main inspiratory muscle, the diaphragm. This network is disrupted in cervical Spinal Cord Injury (cSCI), causing severe respiratory complications, which are the leading cause of morbidity and mortality after cSCI. Previous research has established that while cEINs are not required for normal breathing, they are critical in sustaining breathing post spinal cord injury, and stimulation of these neurons following traumatic injury can rescue breathing during the acute stage. This study aims to investigate the role of cEINs in response to acute respiratory demands such as hypercapnia in both health and after SCI. We hypothesize that cEINs are critical in modulating breathing in conditions requiring greater ventilatory demand.

Methods:
To examine the role of cervical excitatory neurons in promoting breathing after SCI and in hypercapnic conditions, we injected AAV-FLEX-PSAM–GlyR.GFP in the ventromedial area of C3-5 spinal levels of Vglut2::cre mice two week prior to SCI to transiently silence these neurons. SCI was modeled in the form of C2 hemisection. Change in frequency and tidal volume were monitored using whole body plethysmography 4 weeks after surgery.

Results:
Using chemogenetics and whole-body plethysmography, we show that cEINs play a vital role in modulating breathing and increasing ventilatory output in hypercapnic conditions. Silencing cEINs in uninjured mice led to a nearly ten-fold decrease in tidal volume in response to hypercapnia when compared to vehicle-treated controls, illustrating cEIN importance in regulating breathing in response to acute respiratory stress. SCI animals did not have significant decrease in tidal volume after cEIN silencing, indicating that a different mechanism is involved in hypercapnic ventilatory response in injured states.

Conclusion:
The results from the study support the original hypothesis that cEINs are vital in the modulation of breathing during conditions of increased ventilatory demand in health. Additionally, this study revealed there may be differing mechanisms involved in the regulation of acute respiratory stress responses in SCI. Future research could go on to explore which additional circuitry is involved in regulating breathing in hypercapnic conditions after SCI.

Keywords:
Spinal cord injury, cervical excitatory interneurons, whole-body plethysmography, PSAM

Alexis L. Cramer – Middlebury College
Dr. Pui-Ying (Penny) Lam, PhD
Neuroscience Research Center

Investigating the growth rate of Danionella *cerebrum* at different stages of life

Danionella *cerebrum* is a small, translucent fish species found most commonly in freshwater habitats within India. The species is unique in that they lack scales and are translucent into adulthood. This is in contrast to Zebrafish, a phylogenetically similar species and popular neuroscience study model, which are only transparent through the larval stage. D. *cerebrums*' permanent translucent nature allows researchers to obtain an unobstructed view of the species' brain. The species is gaining traction as a study model within the world of neuroscience research due to their translucent body. Despite their emerging popularity, little is known about the species as a whole. This includes their growth rate and life stage timeline. The lack of knowledge reduces researchers' abilities to perform pinpointed age studies within the species. In this study, we seek to determine the growth rate of D. *cerebrum* which would allow for a new wave of targeted, age-specific studies. In order to accomplish this, five different-aged cohorts (n_{female}=8-12, n_{male}=3-6) of D. *cerebrum* (1-month-old, 5-month-old, 8-month-old, 12-month-old, and 17-month-old) were visualized with a Nikon SMZ18 microscope and imaged with a SPOT RT3 camera. Further, each fish was weighed with an analytical scale. ImageJ, an image processing program, was then used to measure the individual body length (cm) and eye diameter (cm) of the fish. The body length, eye diameter, and weight (mg) of each fish were measured once a week and tracked over the course of five weeks. The data collected suggests that eye length, body length, and weight in female D. *cerebrum* increased up until the 12-month-old cohorts. Male D. *cerebrum* showed slightly more variation within their growth. Male body length and weight were only shown to have significant growth at 5 months old while eye diameter was shown to have significant growth up until 12 months old. The variation within the male measurements could be attributed to the small sample within each cohort. In the future, studies with a larger sample would be recommended to make conclusive data. The current data suggest that female growth and male eye diameter growth slows down around 12 months old while the body length and weight of male growth slows down around 5 months. The establishment of growth rate within D. *cerebrum* opens the door for future age-specific research to be performed and improvement of experimental design.

Keywords: Danionella Cerebrum, Neuroscience, Growth Rate, Age

Rodney Willoughby

Marchant Lab

SPUR Abstract

Schistosomiasis is a tropical disease caused by the parasitic flatworm *Schistosoma mansoni* that affects 200 million people worldwide. Praziquantel has been the sole drug used for 40 years to treat schistosomiasis, but its mechanism remains undefined. Since *Schistosoma mansoni* does not survive well outside of a host and is difficult to genetically modify, I tested the free-living flatworm *Macrostomum lignano* to determine if it is a suitable model organism for studying the effects of praziquantel, praziquantel analogs, and alternative antiparasitic compounds. *M. lignano* has already been established as an easily-cultivated organism and is used regularly for genetic manipulation. In the presence of some antiparasitic drugs like praziquantel, *M. lignano* displays a visible phenotype of decreased movement and whole-body contraction while remaining alive, similar to the phenotypic response of *S. mansoni*. To quantify this, I recorded time-lapses of *M. lignano* swimming in solution before and after the administration of a drug, then used tracking software to record the swimming velocity of the worms over time. As expected, *M. lignano* displays a significant decrease in movement after addition of praziquantel. *M. lignano* does not require much effort to maintain and study, so this ease of use and similarity to *S. mansoni* make it a promising model for studying antiparasitic drugs in parasitic flatworms.

Cian Callahan – University of Dayton
Ryuma Tanaka, MD and Takuyo Kanayama MD PhD
MCW Department of Pediatrics: Hematology and Oncology

Single chain variable fragments (scFvs) binding potential to GD2 and B7H3

Background

Chimeric antigen receptors (CARs) are receptor proteins that are engineered to redirect T cells ability to target specific antigens via single chain variable fragments (scFvs) and activate via costimulatory factors. Our previous study showed GD2 and B7H3 as two antigens with positive expression in and high density on pediatric brain tumor cells. Furthermore, both have limited expression on normal healthy cells, with GD2 expression restricted to the CNS, and limited B7H3 expression, which make them optimal targets for CAR T cell therapy. Therefore, the aim of our experiment is to determine scFv binding potential to GD2 and B7H3.

Method

An scFv phage display library from healthy donors was amplified using XL-1 blue cells, isolated, and underwent biopanning. Phage library was added to plated B7H3 and GD2 antigens, washed, and eluted. Phages were reamplified using XL-1 blue cells, and colony number was confirmed. Antigen-specific scFv phages were then isolated and reintroduced to their respective target plated antigens. Panning was repeated a total of four times to increase antigen specificity of scFv phages. At the conclusion of fourth panning, scFv specificity to GD2 and B7H3 were determined using enzyme-linked immunosorbent assay (ELISA).

Results

Successful scFv phage display library amplification and panning were confirmed by positive colony growth. A total of 18 anti-GD2 and 12 anti-B7H3 scFvs candidates from single colonies were tested via ELISA. All anti-GD2 scFvs tested were negative, indicating that scFv phages were not specific to GD2. One anti-B7H3 scFv showed high absorbance in accordance with our dilution ratio, which confirms antigen specificity.

Conclusion

Anti-GD2 scFvs were not obtained. Protocol will be revised, and new strategies will be implemented, to test for anti-GD2 scFvs. Anti-B7H3 scFv was identified and confirmed via ELISA. Sequencing will be performed on the scFv phage to confirm antigen specificity. If positive, scFv will then be cloned and used to construct an anti-B7H3 CAR-T cell therapy.

Keywords: CAR T cell therapy, pediatric brain cancer, single chain variable fragment (scFv), GD2, B7H3

References

1. Pediatric Treatment Editorial Board. “Rare Cancers of Childhood Treatment.” *National Institute of Health: Library of Medicine*, 25 May 2022, https://www-ncbi-nlm-nih-gov.libproxy.udayton.edu/books/NBK65876/.
2. Siegel, David. “Pediatric Cancer Mortality and Survival in the United States, 2001-2016.” *American Cancer Society*, 29 July 2020, https://acsjournals-onlinelibrary-wiley-com.libproxy.udayton.edu/doi/full/10.1002/cncr.33080.

Summer Program for Undergraduate Research 2022
Lauren Wolfrath – Concordia University Wisconsin
Lu Han, PhD
Herma Heart Institute

The effects of transcription factors E2F7 and E2F8 on the ploidy of cardiomyocytes and its suspected role in heart cell regeneration post heart injury utilizing the mouse model

Background

Most somatic cells have the ability to proliferation post maturation; however, cardiomyocytes are in the minority. As a result, cardiovascular diseases' impacts are compounded, lacking a solution to replace failing or dying cardiomyocytes in a human heart post injury.

Mice are able to regenerate heart cells within their first seven days of life (P0 – P7). This gives them the ability to recover and regenerate cardiomyocytes post injury. Our research is attempting to correlate these regenerative properties to cardiomyocyte ploidy. Transcription factors E2F7 and E2F8 inhibit polyploidy. Our hypothesis is that this lack of inhibition of polyploidy via knockout of E2F7/E2F8 will allow cardiomyocytes to maintain their regenerative properties capable of proliferation post injury.

Methods

The mice that were selected for this study consisted of a control group and a group that underwent gene edits utilizing the Cre-loxP recombination system creating either Cre+ (control) or Cre- (gene edited). The mice have either transcription factor E2F7 or E2F8 knocked out. The mice were injected with Edu at P0 and the hearts were harvested at P1. The mice were genotyped from a tail snip.

The hearts were cryogenically frozen, sectioned and then stained with various proliferation and cell cycle markers: Ki67, Phospho-Histone 3, Aurora B The samples were imaged on a confocal microscope. These images were used for quantification of ploidy and nucleation analysis.

Results

The results were determined by comparing quantifications of cardiomyocyte positive and cell cycle positive cells in the Cre+ and Cre- test groups. There was no significant difference in positive cell proliferation signaling between Cre+ and Cre- ($p = 0.35$). This result is as expected due to these hearts being harvested in the regenerative stage for mouse cardiomyocytes.

Conclusion

The mouse model has proven valuable in order to simulate heart conditions and research heart cell regeneration. Our research on transcription factors E2F7/E2F8's role in cardiomyocyte ploidy indicated that in the early stages of life the knockout has no significant effect on cell cycle and proliferation. This was the expected result as the P1 mice are still in the regenerative phase of life.

Key words: heart cell regeneration, cardiomyocyte ploidy, inhibition of polyploidy

Zewei Lei – Lehigh University John
LaDisa, PhD
MCW Department of Biomedical Engineering

Background

Coarctation of the aorta (CoA), is a narrowing of the aorta, resulting in abnormal blood pressure and flow. Coarctation can be detected by the blood pressure gradients in the arms and legs and confirmed by medical imaging. The degree of the pressure gradients across the coarctation determines how soon medical intervention is needed. Severe coarctation of the aorta is often found in the first few weeks of life and requires early surgical intervention, while less severe narrowings may not be discovered until later in life. The current guidelines for treatment for CoA are surgical intervention when the pressure gradient across the coarctation is 20 mmHg. However following treatment CoA patients still exhibit unexpected hypertension despite the similar physical properties after the surgery. The continuing abnormality of the cardiac hypertension indicates potential changes within the walls of blood vessels at the cellular level. For this project, we investigate cellular changes within vessels with no correction, late correction, and early correction of the CoA with different pressure gradients across corectation. We believe that cellular changes occur at a lower pressure gradient than is currently indicated in the surgical intervention which would implicate a need for earlier intervention.

Method

In our research, we focus on the histology of regions of the aorta proximal, distal to the CoA as well as carotid, and femoral arteries. To study the histological changes in the vascular tissues, we use rabbit models to mimic the effects of CoA. We created four distinct levels for pressure gradients across a coarctation 0 mmHg, 5 mmHg, 10 mmHg, and 20 mmHg with three different suture types permanent, dissolvable and rapid dissolvable inorder to mimic no correction, late correction, and early correction of the coarctation. We then sacrificed rabbits and collected vascular tissue inorder to perform immunohistochemistry (IHC) on it. Our project involves five main steps: In-vivo aortic suturing, tissue collection, tissue sectioning, immunostaining, tissue imaging, and image analysis of aortic tissues.

Results

By analyzing the IHC images at each region, we found a significant increase in total medial thickness in both left common carotid artery and proximal regions with apparent elastin fragmentation in the CoA and corrected groups compared with the control group. nonmuscle (NM) myosin staining intensity was significantly increased in proximal arteries of both CoA and corrected rabbits compared with control rabbits smooth muscle (SM) myosin staining intensity of proximal arteries was significantly reduced in CoA and corrected groups compared to the control group whereas no significant differences in SM myosin intensity were found distally.

Conclusion

Our results suggest that CoA can cause phenotypic changes in smooth muscle cells (SMC), as shown by the altered NM and SM myosin expression, which may result in increased medial thickness and stiffness. Under our current surgical protocol, we may not be able to effectively prevent the progression of these abnormalities.

Keywords:
CoA, IHC, SM myosin, NM myosin, SMC, cell differentiation

Suggested references:

Menon A, Eddinger TJ, Wang H, Wendell DC, Toth JM, LaDisa JF Jr. Altered hemodynamics, endothelial function, and protein expression occur with aortic coarctation and persist after repair. Am J Physiol Heart Circ Physiol. 2012 Dec 1;303(11):H1304-18. doi: 10.1152/ajpheart.00420.2012. Epub 2012 Sep 28. PMID: 23023871; PMCID: PMC3532538.