55th Annual Midwest Student Biomedical Research Forum

Saturday, March 2, 2024

P-022 ANALYSIS OF ABDOMINAL ADIPOSE TISSUE QUANTITY, QUALITY, AND WAIST CIRCUMFERENCE USING AQUARIUS INTUITION IN PATIENTS WITH MULTIPLE MYELOMA
Presenter: Ryder Cuppett, UNMC

P-023 CHANGES IN THE HIPPO PATHWAY ARE ASSOCIATED WITH MATRIX REMODELING DURING OVARIAN AGING
Presenter: Dipanwita Das, UNMC

P-026 UNDERSTANDING THE ROLE OF AT-RISK CYTOLOGY AND CO-TESTING CATEGORIES IN CERVICAL CANCER DEVELOPMENT: ARE CURRENT TACTICS IN MANAGEMENT SUFFICIENT?
Presenter: Caroline Doyle, Creighton University

P-027 MIP-BASED PANEL SEQUENCING OPTIMIZATION TO IDENTIFY HEREDITARY CANCER RISK GENOTYPES ACROSS 55 GENES
Presenter: Samantha Draves, Creighton University

P-029 A RETROSPECTIVE REVIEW OF OUTREACH MEASURES IMPLEMENTED AT SHARING CLINIC
Presenter: Emily Evans, UNMC

P-032 DIFFENTIAL MIRNA EXPRESSION IN TACHYCARDIA-INDUCED CARDIOMYOPATHY
Presenter: Braden Fink, UNMC

P-034 QUANTITATIVE PROTEOMIC ANALYSIS TO IDENTIFY PATHWAYS GOVERNING THE BENEFICIAL EFFECTS OF PYK2 INHIBITION IN HYPOXIC CARDIOMYOCYTES
Presenter: Devashri Gandhi, UNMC

P-035 IDENTIFICATION OF NOVEL METABOLIC SIGNATURE IN PANCREATIC CANCER STEM CELL
Presenter: Neelanjana Gayen, UNMC

P-036 OPTIMIZING PROTEIN CRYSTALLIZATION FOR NEUTRON DIFFRACTION AND THE DIRECT DETECTION OF CATALYTIC PROTONS IN CU-ZN SUPEROXIDE DISMUTASE
Presenter: Miles Graham, UNMC

ANALYSIS OF ABDOMINAL ADIPOSE TISSUE QUANTITY, QUALITY, AND WAIST CIRCUMFERENCE USING AQUARIUS INTUITION IN PATIENTS WITH MULTIPLE MYELOMA

[1]Ryder Cuppett, BS, [2]Birgit N. Khandalavala, MD, [2]Jenenne A. Geske, PhD, [3]Jennifer M. Oliveto MD, [4]Tanya M. Wildes MD, MSCI

[1]University of Nebraska Medical Center College of Medicine, Omaha NE USA

[2]Department of Family Medicine, [3]Department of Radiology, [4]Department of Medicine Division of Hematology/Oncology, University of Nebraska Medical Center/Nebraska Medicine, Omaha NE USA

ABSTRACT

Background

Adipose tissue quality, quantity, and waist circumference are associated with multiple myeloma prognosis and outcomes, but their relationship to each other as well as their clinical utility is still being investigated. The purpose of this study was to investigate the relationship between adipose tissue measurements and waist circumference and continue to explore the usefulness of body composition software in multiple myeloma.

Significance of Problem

If radiological imaging can provide accurate insight into the relationship between different adiposity measurements, then obtaining an in-clinic measurement of waist circumference may allow for estimation of adipose tissue density and area and subsequent determination of prognosis related to body composition.

Hypothesis

Higher waist circumference will be directly correlated with less dense subcutaneous and visceral adipose measurements.

Methods

This retrospective study was completed in the United States' Midwest region at a university affiliated hospital. All patients with multiple myeloma were identified by search of electronic medical record diagnosis. Access to subject data and previous patient CT scans was provided by electronic medical records. Patients 19 years of age and older who had whole body CT scans with a listed indication of myeloma skeletal imaging between June 2016 and June 2023 were included. All CT scans were non-contrast and were chosen by the closest date to diagnosis. Aquarius iNtuition was the analytical imaging software used to retrieve adipose tissue measurements. The third lumbar vertebra, with visualization of both transverse processes, was used as a marker to ensure consistent measurement location between scans.

Results

The study included 606 patients, whose mean age was 63 years old and were predominantly white (86%). Using the automated fat analysis tool in Aquarius iNtuition, we found significant correlations, for both men and women, between waist circumference and all the reported adipose tissue measurements reported by the software: subcutaneous adipose tissue area, subcutaneous adipose tissue radiodensity, visceral tissue area, and visceral adipose tissue radiodensity. Larger waist circumference was positively correlated to less dense visceral adipose tissue, a novel finding.

Conclusion

Adipose tissue analysis of CT scans in patients with MM has the potential to impact patient care, and potentially be predictive of outcomes. The relationship between waist circumference and adipose tissue radiodensity in this study also serves to encourage in-clinic waist circumference as an initial prognostic tool, whose accuracy can be confirmed with imaging analysis using similar body composition software. Our future research includes investigation into comparing multiple myeloma staging to the documented adipose tissue measurements.

CHANGES IN THE HIPPO PATHWAY ARE ASSOCIATED WITH MATRIX REMODELING DURING OVARIAN AGING

Dipanwita Das, Kendra Clark, Mahsa Rasekhi, Corrine Monaco, Michele Plewes, Thomas Hopkins, Kim Jonas, John Davis

Departments of Biochemistry and Molecular Biology, Obstetrics and Gynecology, and Cellular and Integrated Physiology. University of Nebraska Medical Center, Omaha, NE ; Department of Women and Children's Health, King's College London, UK

Background: Infertility is one of today's major health concerns affecting approximately 7.5 million women of reproductive age globally. Though there have been medical advancements like IVF (in vitro fertilization) many patients fail to respond to the treatment and undergo multiple IVF cycles. Ovarian aging begins as early as 30 years and peaks diminished fertility by another 10 years. Even with improved medical advancements, older women face challenges to conceive as against young women. One of the major reasons for failure to conceive is the stiffening of ovaries due to senescence. The Hippo signaling pathway is one of the cellular pathways that are associated with ovarian granulosa cell proliferation and follicular development. Hippo signaling is regulated by stiffness due to extracellular matrix components like collagen and fibroblasts, directly affecting the follicular development and proliferation. This pathway consists of multiple components amongst which the Hippo signaling pathway effectors YAP (Yes Associated Protein) and TAZ (WWTR1) play direct role in regulating the expression of various genes via association with the TEAD (Transcriptional Enhanced Associate Domain) family of transcription factors. Although the role of Hippo signaling has been well studied in organogenesis and ovarian tumorigenesis, there is a gap in our understanding of Hippo signaling with respect to ovarian aging and fertility. The current proposal will examine the impact of this pathway in accordance with ageing.

Significance of problem: Women are delaying childbearing until the mid to late 30s when ovarian aging has already begun, thus reducing the chance for successful pregnancies. This demands a better understanding of the physiological and molecular pathways associated with fertility with advanced age in women. Research indicates that the Hippo pathway is a key regulator of follicular growth and steroidogenesis.

Hypothesis: Hippo signaling changes across the ovarian lifespan and changes in Hippo signaling components are associated with alterations in follicular function and development associated with ovarian aging.

Experimental Design: In vivo experiment. We evaluated 4 groups of mice representing various life stages (1) prepubertal: postnatal day 21, (2) young adult: 3-6 months, (3) menopause: 10-14 months and (4) ovarian senescence: 18-20 months. These groups correlate to the various ages and physiology of women ranging from 12-13 years, 20-30 years, 38-47 years and 56-69 years. We performed Western Blot, qRT-PCR and IHC staining analysis of various specific ovarian targets and Hippo signaling pathway components. Serum was screened for the hormones LH, FSH and estradiol. In vitro experiment. We overexpressed YAP or TAZ in a human granulosa cell line followed by analysis of cellular proliferation and analysis of upstream and downstream associated proteins by Western Blot and qRT-PCR.

Results: Body and ovarian weights increased until 10-14 months; ovarian weights were significantly ($P<0.001$) decreased in the oldest group. YAP and TAZ mRNA and protein were observed in follicles and corpora lutea in ovaries at all ages. Based on IHC and mRNA, compared to YAP, TAZ levels were increased in older animals. Levels of LATS1 protein and phosphorylated LATS and YAP proteins were highest in ovaries of prepubertal mice and decreased to low levels in older mice. In mice 10-14 months of age or older, we observed an increase in mRNA expression of *Col1a1* and *Col4a1 and* the YAP/TAZ targets *Ccn1* (CYR61) and *Ccn2* (CTGF). Overexpression of YAP or TAZ in the human granulosa cell line resulted in an increase in extracellular matrix and connective tissue associated proteins like CCN1 and CCN2. Overexpression of YAP or TAZ resulted in differential regulation of *TEAD* mRNA expression. Cell proliferation was significantly increased ($P <.001$) in cells expressing YAP or TAZ compared to control vector.

Conclusions: Aging is associated with a decrease in ovarian weight and increases in expression of extracellular matrix components. Aging is associated with significant changes in ovarian Hippo signaling components, including an elevation in TAZ. Elevation of TAZ in vitro resulted in increased levels of connective tissue associated proteins. Our data suggest that Hippo signaling pathway components and downstream targets play a significant role in ovarian aging.

UNDERSTANDING THE ROLE OF AT-RISK CYTOLOGY AND CO-TESTING CATEGORIES IN CERVICAL CANCER DEVELOPMENT: ARE CURRENT TACTICS IN MANAGEMENT SUFFICIENT?

Caroline Doyle, Dr. Lesley Conrad, Dr. Joyce Pazhayattil, Dr. John Cote, Dr. Emma Bassette, Dr. Gabriela Sotolongo.
Creighton University School of Medicine, Omaha, Nebraska.

Background: HPV infection is the most common cause of cervical cancer worldwide, accounting for nearly 95% of cases. Regular screening is a recommended practice for women 21 years and older. Screening guidelines recommend cytology only for age 21-29, given the high prevalence of HPV presence and clearance in this younger population. Ages 30+ should undergo co-testing (cytology + high risk HPV testing) every 5 years (preferred method) or cytology alone every 3 years. The frequency of patient follow-up screening, and the appropriate treatment, are dependent on cytology/HPV results.

Significance of Problem: Cervical cancer is the fourth most common cancer among women, and is often preventable with HPV vaccination and consistent cervical screening. This burden of disease requires consistent investigation in order to inquire about the cytyology and high risk HPV results that are commonly occuring preceeding a follow-up diagnosis of cervical cancer.

Hypothesis, Problem, or Question: Our project seeks to evaluate at-risk categories of cytology and co-testing results precending cervical cancer diagnoses who may be triaged to a repeat screeing interval over immediate colposcopy based on the current ASCCP guideline recommendations.

Experimental Design: We conducted a retrospective descriptive study of patients diagnosed with cervical cancer from January 2012 to May 2023. Variables collected included: age, race, ethnicity, prior cancer history, tobacco use, hormonal contraceptive use, sexual history, stage and histology of cervical cancer, cervical cancer diagnosis date, preceding cytology/HPV testing results, management after testing, history of cervical dysplasia and treatment types, and cervical cancer treatment and outcomes. Eligible patients identified utilizing the most recent screening test (cytology/HPV testing) preceding their confirmed cervical cancer diagnosis based on 4 at-risk categories: 1) NILM without cytology, 2) NILM with HR HPV non-16/18, 3) ASCUS with HR HPV non-16/18, and 4) LSIL with HR HPV non-16/18 . Exclusion criteria included inconclusive HPV/cytology testing, inconclusive diagnosis, or most recent cytology >1 or 3-5 years (i.e. outside of screening window) from prior to cancer diagnosis.

Results/Data: 127 patients with a cervical cancer diagnosis and available cytology/co-testing were identified from our cancer registry. 31 patients met inclusion criteria (Table 1). Twenty-three (18.1%) patients had NILM cytology preceding a cervical cancer diagnosis, with the majority (60.8%) of patients in the age 30-65 category. An additional 10.1% (7 patients) were older than age 65. Seven (30.4%) patients were diagnosed with early-stage cervical cancer; however, the majority (69.6%, 16 patients) had advanced disease at diagnosis. Ten (43.5%) patients had squamous cell histology, 10 (43.5%) patients had adenocarcinoma, 2 (8.7%) patients with neuroendocrine carcinoma, and 1 (4.3%) patient had adenosquamous histology. In the NILM with HR HPV 16/18, two patients in the age 30-65 category developed cervical cancer. Both patients had HPV 16 positive early-stage squamous cell carcinoma. No patients were identified in the NILM with HR HPV non-16/18 category. In regard to low-risk cytologies, 4 patients ages 30-65 developed cervical cancer in the ASCUS with HR HPV non-16/18. All patients had early stage disease and squamous cell histology. Lastly, two patients ages 30-65 in the LSIL with HR non-16/18 cytology developed cervical cancer. Both patients had squamous cell histology, and 1 patient had early-stage disease while the other had advanced-stage disease.

Conclusions: Preliminary findings emphasize that a cytology alone screening strategy may be insufficient for identification of pre-invasive or invasive disease, particularly in ages 30-65. In addition, when cervical cancer was identified, it was in the advanced stages in over two-thirds of the patient cohort. Incorporation of additional eligible patients and clinical variables in analysis is underway.

MIP-BASED PANEL SEQUENCING OPTIMIZATION TO IDENTIFY HEREDITARY CANCER RISK GENOTYPES ACROSS 55 GENES

Authors: Samantha Draves, Lavanya Uppala, Cynthia Watson, Holly A. Feser Stessman (Creighton University School of Medicine, Omaha, NE)

Background- Family history of cancer or early age of onset are known risk factors for developing cancer in the immediate family, which often has a genetic predisposition terming it hereditary cancer. Oftentimes, *BRCA1* and *BRCA2* mutations associated with breast cancer come to mind when considering hereditary cancer. However, many other genes predispose individuals to neoplasia, including those in tissues related and unrelated to the breast such as colonic, gastric, bladder, ovarian, endometrial, prostate, and pancreatic. Strides in genetic sequencing using molecular inversion probes (MIPs) have now made it more affordable for genetic sequencing in cancer patients and high-risk individuals, where many genes are simultaneously sequenced from one genetic sample. However, the problem persists in identifying who is responsible for determining further care of patients who have tested negative on previous gene panels.

Significance of Problem- A lack of identifiable cause of hereditary cancer can lead to inappropriate screening or surveillance in those with cancer or their family. In turn, this may delay prophylactic treatments that improve patient prognoses.

Hypothesis, Problem, or Question- Families in the Lynch Cancer Collection and Biorepository without a confirmed genetic diagnosis and with a high suspicion for hereditary cancer, when panel sequenced, have an increased chance of identification of high-risk genes with the 55 gene MIP method.

Experimental Design- 55 Genes were selected based on known cancer risks, many of which are in the ClinVar database and have putative documented pathogenicity of mutations. 3,624 MIPs were designed to amplify genetic sequences of specific DNA molecules and hold a unique identifier for the individual the sample belongs to, allowing many genes with multiple samples from separate individuals to be analyzed at once. Optimization requires a baseline analysis of the performance of the MIPs to balance the MIP library, where overperformers and underperformers can be identified. Ideally, all MIPs library should produce sequencing depth within a range of 1.5 standard deviations on a logarithmic scale, which indicates performance.

Results/Data- Initial runs with 1x concentration of MIPs revealed nearly half 1,733 of 3,624 genes performed well, with 1,120 performing at a low level and 771 not performing at all. This led to a 30x spike-in of underperforming MIPs that was completed as a split sample with standard PCR and GC enhanced PCR master mixes that had similar effects on improving underperformers, but the use of GC buffer increased the range of performers. The final optimization to balance performance included a 10x spike-in of MIPs underperforming in the initial 1x run. Analysis of the 10x run revealed 2,625 of 3,624 genes performed well, with 304 performing at a low level and 695 not performing at all. Of note, 6 genes had a high percentage of MIPs that did not perform across all runs.

Conclusions- The use of MIPs for panel sequencing shows initial promise as an effective use of time, money and resources to screen for mutations in genes that predispose an individual to cancer. All hits should be confirmed with Sanger validation. For genes that consistently underperform, other sequencing methods should be pursued when a high clinical suspicion for one of these genes arises.

Title: A RETROSPECTIVE REVIEW OF OUTREACH MEASURES IMPLEMENTED AT SHARING CLINIC

Emily Evans, Shalmali Mirajkar, Jay J. Byrd, Currey M. Zalman, Margarita I. Pipinos, Jenenne Geske, Melanie S. Menning
University of Nebraska Medical Center, Omaha, NE

Background: Student-run, free healthcare clinics (SRFCs) seek innovative and cost-effective outreach strategies to recruit new patients and raise awareness of their services. The current literature presents several approaches to increase community engagement, but the formal evaluation of these outreach efforts is scarce.

Significance of Problem: Two common barriers to community outreach include limited funding and variable student and faculty time commitment. As such, SRFCs are tasked with finding creative ways to implement innovative outreach efforts that target their local communities, recruit patients, and advertise their services in a time- and cost-effective manner. Evaluating these efforts can result in consolidating time and monetary resources so that SRFCs can practically use their funding and student volunteers.

Hypothesis/Problem: The present study aims to measure the effectiveness of outreach efforts implemented over the past year at an SRFC in Omaha, Nebraska.

Experimental Design: A retrospective review of clinic traffic and outreach efforts deployed at our SRFC for 18 months. Six formal outreach methods were identified and included in the analysis. Patient recruitment was measured by calculating the difference in total patient volume 30 days before and after the implementation of an outreach effort. An effort's efficacy was evaluated by calculating the financial expenses and time spent per patient recruited for the corresponding outreach methodology.

Results: Two-thirds of the efforts resulted in increased patient volume, with the additional third being associated with decreased traffic. The most successful outreach effort (Bridge to Care Refugee Health Fair) which resulted in an average increase of 9 patients, only required 0.67 hours of time to be invested per patient, and no monetary investment. The least successful effort (Postcard Campaign) required 2 hours and $11 of investment per patient.

Conclusion: This study provides an approach to evaluate the efficacy of outreach efforts to increase patient recruitment at our SRFC. The financial expenditure, volunteer time, and corresponding efficacy of previous efforts should be considered prior to their re-utilization. Additionally, these findings support future prospective tracking of patient recruitment and retention data to optimize the quality and quantity of patient care. By addressing these challenges, clinics can better serve their target population and fulfill their mission of providing quality medical care to underserved communities.

DIFFENTIAL MIRNA EXPRESSION IN TACHYCARDIA-INDUCED CARDIOMYOPATHY

Authors: **Braden Fink**[1], David Liescheski[1], Sai Vikram Alampoondi Venkataramanan[1], Hesham Basma[1], Kajari Dhar[1], Jason Payne,[1] Brian Lowes[1]

[1] Department of Cardiovascular Medicine, University of Nebraska Medical Center, Omaha, Nebraska

Background: Tachycardia-induced cardiomyopathy (TIC) is a form of non-ischemic dilated cardiomyopathy which is reversible upon resolution of tachycardia. TIC can be induced by various tachyarrhythmias, including atrial fibrillation (AF) and ventricular arrhythmias, as well as from pacing via cardiac implantable electronic devices. To date, little is known about how alterations in RNA biology contribute to the pathogenesis of TIC. Micro RNAs (miRNAs) are small, non-coding RNAs that function to regulate gene expression. By binding to the 3′-untranslated region (3′-UTR) of mRNAs, miRNAs can downregulate gene expression either by blocking mRNA translation or by promoting mRNA degradation through deadenylation.

Significance: Due to the presence of tachyarrhythmias in various cardiomyopathies, differentiating TIC from other non-reversible conditions can be challenging. Investigating miRNA changes in TIC could aid in developing novel therapies and serve as biomarkers, aiming to reduce heart failure and transplantation burdens.

Hypothesis: Rapidly pacing human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CMs) can create an in vitro model of TIC in which to characterize changes in miRNA expression.

Design: iPSC-CMs were cultured in a 24-well plate with 12 serving as controls and 12 paced at 2Hz (120 bpm) for 72 hours using a microelectrode array. Assessments for field potential, propagation, and contractility assays were conducted over the 72-hour period. RNA was isolated via RNeasy® Mini Kit and quantified using the Qubit 2.0 Fluorimeter. Profiling of miRNA and mRNA expression was conducted with the Nanostring nCounter System. Differentially expressed genes ($p<0.05$, ≥2-fold change) were identified using Bioconductor's Linear Models, then assessed for significant biological processes via Ingenuity Pathway Analysis.

Results: After 72 hours, paced iPSC-CMs showed increased field potential duration corrected for heart rate (FPDc) and a steeper increase in conduction velocity compared to controls (slope of 0.00245 vs 0.00147; p value=0.01). Paced iPSC-CMs displayed decreased spike amplitude, beat width mean, and beat period ($p=0.01$, 0.001, 0.001). Among 125 differentially expressed miRNAs ($p<0.05$) in paced cardiomyocytes, 12 showed a fold change > 2. Specifically, miR-16-5p, miR-291a-3p, miR-205-5p, and miR-520g-3p were upregulated in paced cells, correlating with reduced VEGFA mRNA expression. Furthermore, the upregulation of miR-443 and miR-6721-5p correlated with downregulated TNNC1 and MYL3 mRNAs.

Conclusion:
Tachycardia pacing of iPSC-CMs produces an in vitro model mimicking TIC with electrical and contractile abnormalities. Tachycardia pacing induces changes in miRNA expression that contribute to the pathophysiology of heart failure. This model of TIC can be utilized to better understand the molecular mechanisms of this specific cardiomyopathy and identify potential biomarkers or novel targets for therapy.

QUANTITATIVE PROTEOMIC ANALYSIS TO IDENTIFY PATHWAYS GOVERNING THE BENEFICIAL EFFECTS OF PYK2 INHIBITION IN HYPOXIC CARDIOMYOCYTES

Devashri Gandhi[1], Kanika Sharma[1], Paul Sorgen[2]

[1]Department of Genetics, Cell Biology and Anatomy, [2]Department of Biochemistry and molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska.

Background:

Heart failure, a prevalent cardiovascular disorder, imposes a substantial global health burden. Pyk2, a non-receptor tyrosine kinase, plays a pivotal role in the pathophysiology of heart failure, with its phosphorylation linked to maladaptive myocardial remodeling and impaired cardiac function. Particularly under hypoxic conditions, Pyk2 activation exacerbates adverse effects on the heart, compromising contractility and increasing susceptibility to arrhythmias.

Inhibition of Pyk2, facilitated by the Pyk2 inhibitor PF4618433, demonstrates promise in alleviating the consequences of Pyk2 phosphorylation in heart failure. Previous studies in animal models highlight the favorable effects of Pyk2 inhibition, indicating improved cardiac function and reduced susceptibility to heart failure. While Connexin43 has been identified as a key mediator in maintaining cell-to-cell communication, its role alone does not fully explain the observed enhancements in cardiac function.

Hypothesis:

Building upon this background, our hypothesis proposes that Pyk2 inhibition with PF4618433 intervenes in phosphorylation-mediated pathways leading to heart failure. Anticipating that the Pyk2 inhibitor not only mitigates the effects of hypoxia on cardiac function but also modulates key pathways in cardiomyocytes associated with heart failure, this study aims to unravel specific molecular mechanisms underlying the protective effects of PF4618433. The objective is to extend our understanding beyond the known role of Connexin43.

Experimental Design:

The experimental design integrates tandem mass spectrometry to acquire precise quantitative data on neonatal rat ventricular myocyte samples. Acknowledging limitations in traditional bioinformatics methods, tandem mass spectrometry is combined with Weighted Gene Co-expression Network Analysis (WGCNA). This synergistic approach enhances interpretability of regulatory networks, facilitating the identification of biologically meaningful modules and hub proteins.

Employing neonatal rat ventricular myocytes in a hypoxia model, the study compares those treated with PF4618433 to those without, utilizing tandem mass spectrometry and novel bioinformatics analysis. The focus lies in identifying hub proteins associated with non-Connexin pathways, elucidating broader mechanisms contributing to the protective effects of PF4618433 in hypoxic conditions and preventing heart failure.

Conclusion:

This comprehensive investigation enhances our understanding of cardiac intercellular communication and sheds light on the intricate Pyk2-mediated pathways leading to heart failure. The integration of advanced analytical techniques with an inhibitor-based approach provides a holistic view of the molecular networks affected by Pyk2 inhibition, offering promising therapeutic avenues for addressing heart failure and associated complications.

IDENTIFICATION OF NOVEL METABOLIC SIGNATURE IN PANCREATIC CANCER STEM CELL

N. Gayen, N. Krishna Kumar[1], A.O Ogunleye[1], K. Balakrishnan[1], S.K. Batra[1], and M.P. Ponnusamy[1].

[1]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE.

Background and Significance: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with a poor prognosis. Recent studies have highlighted the crucial role of cancer stem cells (CSCs) in the initiation, progression, drug resistance, and recurrence of PDAC tumors. CSCs are a small subpopulation of cells within tumors that have the capability to self-renew, differentiate, and generate tissue to propagate the tumor. CSCs exhibit distinct metabolic phenotypes and an incredible capacity to adapt their metabolism to survive under various stressful situations, which contributes to chemo-resistance, metastasis, and disease relapse. In this study, we aim to investigate unique signatures that regulate the metabolic alterations in CSCs. Understanding specific signatures that regulate these metabolic alterations in CSCs will pave the way for developing novel therapeutic strategies targeting CSCs and improving patient outcomes.

Hypothesis: *Pancreatic cancer (PC) stem cell maintains distinct metabolic reprogramming and plays an important role in the progression of pancreatic ductal adenocarcinoma.*

Experimental Design/Methods: We developed a sphere culture (stem cells) model using SUIT2 cells. RNA sequencing was performed in SUIT2 control and spheres, iPSC, and HPNE cells, to identify differentially expressed genes in the CSCs enriched sphere population compared to the control**.** Top differentially expressed putative metabolic genes were selected. SUIT2, SWI990, and MiaPaCa-2 PC cells were grown in sphere culture with stem cell conditions to isolate the CSC population. Also, the side population (SP/CSC), non-side population (NSP/non-CSC), CD133+ve, and CD133-ve populations were isolated using flow cytometry. The expression of the selected metabolic genes was further evaluated *in vitro* using various techniques, including qPCR, dd-PCR, Western blotting, Immunohistochemical staining, and Immunofluorescence microscopy. Additionally, Dual Immunohistochemical staining was carried out using human pancreatic tumor tissues to investigate the co-expression between the selected metabolic signature and the stemness markers. Lentiviral Knockdown (KD) was used to analyze the effect and changes in stemness status in normal pancreas and pancreatic cancer cell lines.

Results: In our *in-silico* analysis, we identified 14 over-expressed putative metabolic regulatory genes. Following extensive *in vitro* validation, one of the top selected genes, PDK3, was found to have significantly increased mRNA and protein expression in pancreatic CSCs compared to their parental counterpart. Histological analysis revealed that PDK3 expression was higher in human pancreatic cancer tissue (overexpressed in specific cell types) than in normal human pancreas tissue. Intriguingly, suspension tumor spheres developed from PC cells SUIT2, SWI990, and MiaPaCa-2 showed an increased expression of PDK3 compared to control cells. Furthermore, PDK3 showed increased expression in other stemness populations like SP and CD133+ve compared to their non-stem cell counterparts. Dual IHC staining revealed that PDK3 was co-localized with the stemness marker CD44. A lentiviral-mediated knockdown of PDK3 in SUIT2 revealed that the stemness features in the KD population were reduced in both protein and mRNA.

Conclusions and Future Perspectives: Our results demonstrate that the pancreatic CSCs sub-type population expresses the distinctive metabolic regulatory gene PDK3, indicating a unique metabolic reprogramming in these cells. Further mechanistic studies on PDK3 and analysis of changes in stemness are required to establish its role as a potential metabolic regulator in PC.

OPTIMIZING PROTEIN CRYSTALLIZATION FOR NEUTRON DIFFRACTION AND THE DIRECT DETECTION OF CATALYTIC PROTONS IN CU-ZN SUPEROXIDE DISMUTASE

Miles Graham, Jahuan Azadmanesh, Lucas R. Sturble, Gloria E. O. Borgstahl
University of Nebraska Medical Center, Omaha, NE

Background

Human Copper-Zinc Superoxide Dismutase (CuZnSOD) is a cytosolic metallo-oxidoreductase whose function is to scavenge superoxide radicals. Left unchecked, these unstable superoxide radicals may react with and damage vital cellular components resulting in oxidative stress and potentially cell death. CuZnSOD regulates the concentration of these free radicals by converting the unstable superoxide into hydrogen peroxide and molecular oxygen via the following reaction.

$$Cu^{2+} + O_2^{\cdot -} \rightarrow Cu^{+} + O_2$$
$$Cu^{+} + O_2^{\cdot -} + 2H^{+} \rightarrow Cu^{2+} + H_2O_2$$

The copper is reduced and then, with the aid of a proton transfer, oxidized during a full catalytic cycle.

Significance

CuZnSOD function is critical cellular homeostasis and the prevention of oxidative stress. Loss of redox homeostasis has implications in cancers, autoimmunity, and neuropathies such as ALS. Despite its clinical significance of CuZnSOD's function, the precise molecular mechanism by which it performs the aforementioned reactions is still unknown. The elucidation of this mechanism is of critical importance as it may be able to shed light on how redox homeostasis is maintained, and how dysregulation can occur.

Hypothesis

Based on the recently uncovered reaction mechanism of a similar metallo-oxidoreductase, human manganese superoxide dismutase (MnSOD), we hypothesize that the metal in the active site of CuZnSOD causes the neighboring amino acids to have unusual pKas that facilitate a concerted proton and electron transfer mechanism to achieve fast and efficient catalytic action. We will use neutron crystallography to structurally resolve this mechanism critical proton in both oxidized and reduced states. To this end, a pipeline for growing large, perdeuterated CuZnSOD crystals must be developed

Experimental Design

We expressed CuZnSOD intracellularly in BL21(DE3) *E. coli.* $CuSO_4$ doped cell lysate was purified using ammonium sulfate precipitation, hydrophobic interaction chromatography, and ion exchange chromatography. Purified CuZnSOD at a concentration of 10mg/ml was tested for catalytic activity and then crystalized in a solution of ammonium sulfate and NaCl using the sitting drop method. Crystals were allowed to grow for 2 months before being used for X-ray diffraction imaging under cryogenic conditions.

Results

Purified CuZnSOD was shown to be catalytically active in a qualitative NBT assay. Our crystallographic conditions provided large, individual, 3-dimensional crystals. X-ray diffraction data was collected to 3.22Å that the crystals were in the P6322 space group with unit cell dimensions of 243Å x 243Å x 144Å. The asymmetric unit was found to contain 9 homodimers giving a total of 18 active sites per asymmetric unit.

Conclusion

The ease with which active CuZnSOD can be purified from *E. coli* shows that production of fully perdeuterated protein is highly feasible. The morphology of the crystals was encouraging, with the relatively large size and 3-dimensional growth indicates that crystals large enough for neutron diffraction could be grown given more time and protein. The 3.22Å resolution we collected is too low to be usable for our work, but higher resolutions reported in literature for similar crystals indicate this was likely just an issue with the individual crystal selected for data collection. The high symmetry space group was also encouraging because more symmetrical reflections increase data redundancy and reduces the beam time necessary to collect a complete data set. This reduction in beamtime is counteracted by the large unit cell, but it is still within the collection capabilities of current neutron scattering equipment like MaNDi, the world's most sophisticated macromolecular neutron diffractometer, that is capable of handling unit cell lengths of up to 300Å. Overall, our results show that neutron crystallography, and the functional elucidation of one of the key cellular antioxidants, is close on the horizon.