55th Annual Midwest Student Biomedical Research Forum

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Authors: Anna A. Haggart, Joshua F. Baker, Yangyuna Yang, Michael Duryee, Geoffrey Thiele, Ted R. Mikuls, Bryant R. England

School: University of Nebraska Medical, Omaha, NE 68198

Title: ADIPOKINES AND INTERSTITIAL LUNG DISEASE IN RHEUMATOID ARTHRITIS

Background and Significance

Rheumatoid arthritis (RA) is a systemic inflammatory disease that manifests primarily as an inflammatory arthritis; however, pulmonary involvement in the form of interstitial lung disease (ILD) is a common extra-articular manifestation, that carries a substantial increase in mortality risk among RA patients.

Because of its dramatic impact on survival, there is an ongoing need to improve the early identification of RA-ILD. The most clinically relevant peripheral biomarkers in RA are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), however, while RA patients with these autoantibodies were more likely to have prevalent ILD, RF and ACPA, seropositivity was not strongly associated with incident RA-ILD. Thus, there remains a need to identify novel biomarkers that are predictive of RA-ILD.

Adipokines are hormones involved in the regulation of metabolism and energy homeostasis and play a role in the promotion of energy metabolism. Adipokines have been shown to be linked with poor long-term outcomes in RA including higher rates of all-cause mortality and cardiovascular events. Adipokines have also been associated with idiopathic pulmonary fibrosis (IPF), a disease sharing histo-radiologic overlap with RA-ILD. The potential relationship between adipokines and incident RA-ILD is not yet well investigated.

Objective and Hypothesis

The objective of this study is to assess whether elevated levels of adipokines including adiponectin, leptin, and FGF 21, may be associated with prevalent RA-ILD as well as the development of incident RA-ILD. We hypothesize that higher levels of circulating adipokines will be associated with higher prevalence and incidence of RA-ILD.

Experimental Design

We conducted both a cross-sectional study of prevalent ILD and a cohort study of incident ILD among participants within the Veterans Affairs Rheumatoid Arthritis (VARA) registry, a multicenter, prospective cohort of U.S. Veterans with RA. Adipokines were measured from banked serum samples from enrollment using the MesoScale Discovery platform and analyzed as log-transformed, standardized continuous values and values dichotomized above/below the median value. ILD diagnoses were validated via medical record review by a rheumatologist specializing in RA-ILD requiring clinical diagnosis supported by either chest imaging or lung biopsy findings. Logistic regression models quantified associations between prevalent ILD and adipokines while multivariable Cox models were used to assess associations with incident ILD, adjusting for potential confounders. Covariates included age, sex, race, smoking status, body mass index (BMI), serologic positivity for autoantibodies (RF, anti-CCP) and comorbidities via Rheumatic Disease Comorbidity Index scores (RDCI).

Results

Among 2,673 participants (mean age 64 years, 88% male), 112 (4.1%) had prevalent ILD at enrollment. There was a non-significant trend towards higher adiponectin concentrations being associated with prevalent ILD (odds ratio [OR] per 1 SD increase: 1.19 [95% CI 0.95-1.49], P = 0.12). Leptin and FGF 21 were not associated with prevalent ILD (p value >0.40). Exploratory analysis of associations of forced vital capacity (FVC) with adipokine concentrations did show a trend towards association of lower FVC with adiponectin concentrations above the cohort median value (p = 0.08). Among 2,668 participants without prevalent ILD, 128 (4.7%) developed incident ILD over 18,479 patient-years of follow-up. There were no significant associations between adiponectin, leptin, or FGF-21 concentrations with incident ILD risk (hazard ratio [HR] per 1 SD increase: range 0.91 to 1.04; p >0.42). Findings were consistent in several subgroup and sensitivity analyses.

Conclusions

Serum adipokine concentrations were not significantly associated with prevalent or incident ILD and are unlikely to meaningfully improve RA-ILD risk stratification. Results from exploratory analyses suggesting a potential relationship of adiponectin with FVC impairment could indicate that adipokines may confer prognostic value in RA-ILD. Further study is needed to explore the relationship between RA-ILD and metabolic health and to identify tools for early identification or disease prognostication.

EPIGENETIC MODIFICATIONS BY FLT3-ITD MUTATION IN PEDIATRIC ACUTE MYELOID LEUKEMIA (AML)

Lauren Jacobsen**,** Ben Nolan, Dr. Christopher Cummings, Dr. Jordan Rowley

[1]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198
[2]College of Medicine, University of Nebraska Medical Center, Omaha, NE

Background: Acute myeloid leukemia (AML) is a hematopoietic malignancy in children and the leading form of acute leukemia in adults. Current interventions are associated with a five-year survival rate of 60-70% for pediatric patients, necessitating the development of additional therapeutics to improve survival and prevent disease recurrence. A broad scope of somatic mutations has been suggested to contribute to AML pathogenesis. However, variants in *FLT3* receptor tyrosine kinase with internal tandem duplication (ITD) and D835 point mutations are present in many AML cases. *FLT3*-ITD leads to constitutive activation of receptor tyrosine kinase activity leading to uninhibited growth of AML cells, as well as indirectly driving epigenetic modifications to secondarily drive other pro-oncogenic pathways. Therapies directly targeting *FLT3*-ITD have some improved patient outcomes, but responses are not curative.

Significance of Problem: This research aimed to delineate any other secondary pro-oncogenic or anti-apoptotic gene expression networks that could serve as areas to expand targeted AML therapy.

Hypothesis: We hypothesized that *FLT3*-ITD mutations in AML cell lines would drive alterations in chromatin accessibility and histone modifications, resulting in aberrant gene expression associated with pro-oncogenic pathways.

Experimental Design: We used ChIP-seq and ATAC-seq to analyze histone modifications and chromatin accessibility, respectively, in DNA from MV411 and Kasumi AML cells treated with *FLT3* inhibitor, Gilteritinib, or vehicle control. We assessed four core histone marks: H3K27ac, H3K4me1, H3K27me3, and H3K9me3. H3K27ac and H3K4 me1 are associated with active chromatin states, specifically active and putative enhancers, respectively, while H3K27me3 and H3K9me3 are considered repressive marks. This allowed us to identify any subset of modifications that were dependent on *FLT3*-ITD activation and were reversed in AML cell lines treated with the inhibitor, Gilteritinib. In-house bioinformatics pipelines used read alignment and peak calling to analyze differential histone occupancy and chromatin accessibility.

Results: Several differential binding sites were identified for each of the histone modifications assessed in initial studies, and a repeat of these is currently being assessed. The Gilteritinib-treated Kasumi cell line and the Gilteritinib-treated MV411 cell line both had a subset of differential peaks with the H3K27ac, H3K27me3, H3K4me1, and H3K9me3 histone marks. The differential peaks found in the first samples will be compared against the replicates, which are currently in the sequencing core. The analyzed differential peaks will be linked to the differential gene expression and pathway analysis to identify any novel transcription networks that are governed by epigenetic modifications induced by *FLT3* activation. ATAC-seq studies have additionally been performed and are currently undergoing bioinformatics analysis.

Conclusions: This project showcased that histone marks are broadly changed in the setting of FLT3-activation, and these are revealed with the inhibitor Gilteritnib. These changes are promising targets for future therapies and can serve as a foundation for future research projects to tie histone changes to gene transcription changes.

VACCINE DISPARITIES IN A SYSTEM LACKING FINANCIAL AND ACCESS BARRIERS

Nansea M. Ji, Kelsey L. Witherspoon, Marvin J. Bittner, Amanda C. Yeates, Raul Isern, Jennifer Zimmerman
Creighton University, Omaha, Nebraska

Background
A highly effective method to prevent and mitigate infectious diseases is vaccine administration. However, there are many barriers that keep vaccine uptake rates at suboptimal levels, including split public opinion on the safety and efficacy of vaccines, financial concerns, and accessibility.

Significance of Problem
Increased vaccination rates would greatly reduce morbidity and mortality rates from infectious diseases. Some of the populations that are most vulnerable to infectious diseases such as historically marginalized racial and ethnic groups have the lowest rates of vaccination. This problem has been recognized for years, but disparities in vaccine uptake have persisted and new strategies are needed to address them.

Question
Notably, a quality improvement project done at Omaha primary care clinics of the Veterans Health Administration Nebraska-Western Iowa Health Care System demonstrated that there was no racial/ethnic disparity in the receipt of the initial COVID-19 vaccine, whereas receipt of the zoster vaccine in this same patient population did have racial/ethnic disparities. Our study aimed to characterize the patients from the quality improvement project and identify potential reasons why the racial/ethnic disparities in vaccine uptake disappeared in the receipt of the initial COVID-19 vaccine but not the zoster vaccine.

Experimental Design
This was a retrospective study of the same patient population from the quality improvement project. These patients do not face financial or healthcare access barriers to vaccinations, which are two factors known to lower vaccination rates. In absence of these barriers, we analyzed other factors that may influence vaccine uptake. A random sample of 400 patients seen during the same time frame as the quality improvement study was chosen. To be chosen, the patient had to be eligible for both the zoster and COVID-19 vaccinations at the time of their visit. We examined their electronic health records for factors including age, address, race/ethnicity, vaccination status against COVID-19 and zoster, provider characteristics, and number of visits with their provider.

Results/Data
Our study population was 82.3% non-Hispanic White (NHW), 11.5% non-Hispanic Black (NHB), and 1.3% Hispanic White. 28.8% were visiting their provider for the first time, and 51% of patients had one visit with their provider during the study's time frame. 50.2% of NHWs and 21.7% of NHBs had received both doses of the recombinant zoster vaccine, whereas 86.3% of NHWs and 91.3% of NHBs received an initial COVID-19 vaccine. 39.5% of the NHWs and 71.7% of the NHBs received the COVID-19 vaccine but not the zoster vaccine. 88.7% of patients with one visit to their provider and 91.4% of patients who had more than one visit with their provider received either vaccine. 90.2% of patients with shorter than one year relationship with their provider and 89.7% of patients with over a year relationship with their provider had received either COVID-19 or the zoster vaccine. Among individuals seen by non-resident providers, there was no relationship between racial concordance and the receipt of any vaccine (57.7% regardless of concordance).

Conclusions
Our study was consistent with the quality improvement project in demonstrating that there were racial/ethnic disparities in uptake of zoster vaccine that disappeared for uptake of the COVID-19 vaccine. Our results showed that the provider characteristics (racial concordance, length of the patient's relationship, and number of visits) did not have an association with vaccination status. Further study of other determinants such as age, provider demographics, and geographic factors is ongoing.

THE ASSOCIATION BETWEEN COGNITION AND UPPER EXTREMITY MOTOR REACTION TIME IN OLDER ADULTS: A NARRATIVE REVIEW

Alexandria Jones,[1] Natalie Weaver,[2] Mardon E. So,[2] Abbis Jaffri,[2] Rosalind L. Heckman[2]

[1]School of Medicine, Creighton University, Omaha, NE, USA
[2]Department of Physical Therapy, Creighton University, Omaha, NE, USA

ABSTRACT

Background: Response timing is essential to optimal sensorimotor control across the lifespan. While it is broadly assumed that reaction time increases as cognition declines with age, it is unclear if this assumption is supported by the literature. The purpose of this narrative review was to determine the association between cognition and upper extremity reaction time in older adults. Global cognition and cognitive domains of sensation and perception, motor construction, perceptual motor function, executive function, attention, learning and memory, and language were considered. **Methods**: We conducted a systematic search using Scopus database. The search strategy was designed to meet four inclusion criteria: 1) community-dwelling adults >60 years, 2) upper extremity motor task, 3) at least one cognitive assessment, 4) simple reaction time measure. 1,154 articles were screened. **Results**: Two articles met the full inclusion criteria, but these studies did not associate the cognitive assessment and simple reaction time measures. Nine articles that met three inclusion criteria were reviewed. We found that executive function and learning and memory have been associated with complex and choice reaction time measures. Language, perceptual motor function, and attention have been studied with mixed evidence for an association with reaction time; whereas, sensation and perception and motor construction have not been assessed. **Conclusions**: Overall, limited research has compared cognitive domain function and simple reaction time to determine if age-related changes are associated. While the complex interplay between cognition and motor function is of substantial interest, these measures are often interdependent and additional knowledge is needed to understand their influence on sensorimotor control with age.

LAMA5-MEDIATED CAF-CANCER CELL CROSSTALK PROMOTES PANCREATIC CANCER AGGRESSIVENESS

Annant B. Kaur[1], Seema C. Parte[1], Surinder K. Batra[1], Moorthy P. Ponnusamy[1]
[1]*Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE.*

Background and Significance: Pancreatic cancer is the fourth leading cause of cancer-related deaths in the US, with a dismal 5-year survival rate of 12.5%. The most common type of pancreatic cancer, Pancreatic Ductal Adenocarcinoma (PDAC), often presents with multi-organ metastasis at the time of diagnosis, with limited treatment options and a high mortality rate. A dense desmoplastic tumor stroma is a hallmark of PDAC, often contributing to around 90% of the tumor area in the late stages. PDAC microenvironment comprises various cellular and acellular components, including cancer-associated fibroblasts (CAFs), immune cells, extracellular matrix (ECM), cytokines, growth factors, etc. CAFs (the most abundant cell type in PDAC stroma), secrete many acellular stromal constituents, including ECM proteins like laminin, collagen, etc. Laminin subunit alpha 5 (LAMA5) is an integral part of the laminin heterotrimer and interacts with the cell-surface receptors, such as integrins, to mediate ECM-regulated cellular processes. LAMA5 has been implicated in the proliferation, metastasis, and angiogenesis of various cancers, including colorectal cancer, ovarian cancer, breast cancer etc., but its role in pancreatic cancer progression remains unexplored. Thus, the aim of this study is to investigate the LAMA5-mediated mechanism of initiation and progression of pancreatic ductal adenocarcinoma.

Hypothesis: LAMA5 PROMOTES INITIATION AND PROGRESSION OF PANCREATIC CANCER BY FACILITATING TUMOR MICROENVIRONMENT-CANCER CELL CROSSTALK

Experimental Design: A mass spectrometry-based proteomics analysis of mouse CAF-conditioned media identified top CAF-secreted proteins in PDAC. These proteins were further subjected to gene expression and relative survival analysis via publicly available PDAC RNA seq datasets from TCGA and GEO (number of samples: 804). An amalgamation of *in vitro* and *in vivo* experiments detailed the expression profile of the protein of interest, LAMA5. Protein expression was confirmed in mice autochthonous models of pancreatic cancer progression (KrasG12D/+; Pdx1Cre) and human tumor vs normal samples in human tissue microarray using immunofluorescence (IF) and immunohistochemistry (IHC). To observe the localization of the protein in early PDAC development, a cerulein treatment model was used to induce acute pancreatitis in mice. Since CAFs are the main producers of ECM proteins and are involved in exosome mediated cellular crosstalk, exosomes were isolated from conditioned media of normal and cancer-activated fibroblasts in both mice and human. Exosomal fractions from pancreatic cancer and normal cell lines were also subjected to immunoblot analysis to get a comprehensive view of the CAF-cancer cell crosstalk. Extracted exosomes were confirmed by the presence of exosomal marker proteins such as TSG101, CD63, Hsp70.

Results/Data: Proteomics analysis of mouse CAF-conditioned media identified elevated ECM components, with LAMA5 emerging as one of the significantly overexpressed proteins in the activated fibroblast secretome. Publicly available RNA seq data analysis revealed a significant increase in LAMA5 expression in cancer as compared to normal pancreas, with a high LAMA5 expression correlating with a low survival rate in PDAC patients. IF and IHC analysis of LAMA5 expression revealed a gradual increase in LAMA5 levels with higher PANIN stages and finally PDAC. Cerulein treatment-induced acute pancreatitis in WT and KC mice also drove an enhanced expression of LAMA5 in CAF and epithelial cancer cell compartments. Further, as confirmed by IF, human and mouse CAFs depicted a more prominent LAMA5 expression than normal human fibroblast and mouse stellate cells. An immunoblot analysis of the isolated exosome fractions from normal and cancer-activated fibroblasts revealed substantially elevated LAMA5 protein in the CAF samples of both mice and humans compared to normal fibroblasts. To further characterize this crosstalk, exosomal fractions from different pancreatic cancer cell lines were also analyzed for the presence of LAMA5. Indeed, a high expression of LAMA5 was observed in both primary tumor and metastatic cell lines. However, LAMA5 is constantly overexpressed only in the secreted exosomal fractions compared to the cellular lysates.

Conclusions: Our results show evidence of a potential role of LAMA5 in facilitating intercellular communication between CAFs and cancer cells, thus promoting the aggressiveness of the cancer. LAMA5 may function as a “matrix modulating molecule” to alter the extracellular environment at local and distant metastatic sites in pancreatic cancer.

FOXM1 INHIBITION DOWNREGULATES SMALL CELL LUNG CANCER GROWTH AND METASTASIS

Md Arafat Khan[1], Parvez Khan[1], Mahek Fatima[1], Asad Ur Rehman[1], Ramakanth Chirravuri Venkata[1], Zahraa Wajih I Alsafwani[1], Laiba Anwar[1], Aatiya Ahmad[1], Jesse L. Cox[2], Sung Hoon Kim[3], Benita S Katzenellenbogen[4], John A. Katzenellenbogen[3], Apar K Ganti[5], Surinder K Batra[1,6,7], Mohd Wasim Nasser[1,6,*]

*[1]Department of Biochemistry and Molecular Biology, [2]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE-68198, USA. [3]Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, [4]Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL-61801, USA, [5]Division of Oncology-Hematology, Department of Internal Medicine, VA-Nebraska Western Iowa Health Care System, and Division of Oncology-Hematology, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE-68198, USA, [6]Fred and Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE-68198, USA, [7]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE-68198, USA. *Correspondence E-mail: wasim.nasser@unmc.edu*

Abstract

Small cell lung cancer (SCLC) is one of the deadliest subtypes of lung cancer. Most of the SCLC patients are diagnosed with metastatic disease, and as a result, the overall survival rate is very poor. Platinum-based chemotherapy and etoposide has been used as the primary therapy for >30 years, and the lack of targeted therapies further complicates the disease outcome. SCLC responds very well to first-line chemotherapy; however, relapse occurs rapidly with resistance. Due to the expeditious development of drug resistance, SCLC patients have an average survival of <14 months. For this reason, identification of therapeutic target(s) and targeted therapy for SCLC is an unmet clinical need. The aim of our study is to develop a potential targeted therapy that will hinder SCLC growth, metastasis, and chemotherapeutic resistance. To identify potential targets for drug-resistant SCLC, we have developed cisplatin-resistant SCLC cell lines, and through RNA-sequencing analysis, FOXM1 was identified as one of the most differentially upregulated genes in resistant cells compared to naïve. We inhibited FOXM1 in SCLC cell lines (SBC-3, SBC-5) using FDI-6 and other novel (NB 73 and NB55) FOXM1-specific inhibitors (FOXM1i). It has been found that FOXM1 inhibitors significantly reduced SCLC cell growth and migration. We have further evaluated the efficacy of these FOXM1i alone and in combination with cisplatin in xenograft and spontaneous mouse models and observed that targeting FOXM1 significantly downregulated SCLC growth (and metastasis in spontaneous mouse models). To further understand the associated molecular mechanism, future studies including genetic knockdown of FOXM1 will be performed. The present study suggests that FOXM1 inhibition in combination with cisplatin could be used as a novel combinatorial therapy for SCLC.

ARTIFICIAL INTELLIGENCE IN IDENTIFYING LISFRANC INJURIES

Monty Khela, Alexander Sawatzke, Neil Antonson, John Windle, David Ellis, Peng Dongming

Creighton University School of Medicine – Omaha, NE

Background/Significance of Problem: Lisfranc injuries are acute midfoot injuries involving the bones and/or ligaments in the tarsometatarsal joints. Injuries of the joint can range from complete tarsometatarsal dislocation with associated fractures and ligamentous tears to slight sprains with no displacement. Given that these injuries are commonly missed in Emergency Departments or Family Practice clinics, untreated Lisfranc can lead to degenerative changes such as chronic arthritis, dysfunction, and pain. Therefore, it is essential to identify these injuries radiographically to initiate early treatment and prevent long-term sequelae. The convolutional neural network (CNN) is superior when it comes to image classification since it can take the inputs as a 2-dimensional image dataset with multiple channels. This enhances the capability of a CNN model to retain the spatial features of an image consisting of multiple channels. Due to this feature, the CNN model has been very popular in the current state-of-the-art image classification problem. However, there is limited research employing CNNs to diagnose Lisfranc injuries. Therefore, this study aims to utilize CNNs to better identify Lisfranc injuries on foot radiographs so they are less frequently missed in the clinical setting. The result will be confirmation of Lisfranc injury diagnosis, and in some cases, diagnosis of an otherwise missed Lisfranc injury. This will result in improved patient care and outcomes.

Hypothesis: Convolutional neural network models, trained on a retrospective dataset spanning two decades, will perform to the level of a traditional orthopaedic surgeon in accurately diagnosing Lisfranc injuries.

Experimental Design/Methods: A retrospective review of patients primarily evaluating foot radiographs collected from the electronic medical record. When a radiograph did not clearly indicate Lisfranc injury, medical record review was completed to confirm clinical diagnosis. There is not a predetermined minimum number of images needed to develop a successful model. Based on current projects and generally accepted machine learning principles, 1000 images can develop a meaningful network model. In general, the more images the model has, the more accurate the outcome is.

This was a supervised machine learning project. 1110 images were classified by an Orthopaedic Surgeon and multiple deep convoluted neural networks were modeled to identify the best deep network model for the data set. Accuracy, Sensitivity, Specificity, and Area Under the Curve were utilized as indicators of best deep neural network model. Current studies utilize Inception v3 and Resnet 50 deep networks to identify Lisfranc injuries. This project utilized Inception and Resnet deep neural networks for direct comparison with current literature, as well as other deep convoluted neural networks with adjustment of parameters per data scientist discretion, to identify Lisfranc injuries on both weightbearing and non-weightbearing radiographs with multiple orientations (anteroposterior, lateral, and oblique), which is true to how patients present with these often-subtle injuries. The most accurate deep network model was utilized and improved with more radiographs either across institutions or with future imaging at current institution.

Results: For weight-bearing radiographs, the model achieved an accuracy of 88.4%, indicating a substantial level of correctness in classifying the injury. The area under the curve value was notably high at 93.2%, reflecting great discriminatory power. The dataset for weight-bearing radiographs consisted of 548 cases , and the model demonstrated a sensitivity of 91.3% with a specificity of 67.9%. Similarly, in the analysis of non-weight-bearing radiographs, the model exhibited a higher accuracy of 89.6%, showcasing its effectiveness in correctly classifying the injury. The area under the curve value remained strong at 90.4%, signifying the model's robust discriminatory capabilities. The dataset for non-weight-bearing radiographs comprised of 562 cases, and the sensitivity of the model was 90.7% with a slightly lower specificity at 66.7%.

Conclusion: This study is innovative in the field of orthopaedic surgery and relevant to translational clinical medicine as it aims to confirm and improve diagnosis of Lisfranc injuries known to cause long-term arthritis, sequela, and pain in the midfoot if not identified and treated appropriately. Furthermore, deep convoluted neural networks are effective at identifying these injuries on weightbearing films. Identifying these injuries on non-weightbearing films accurately has not yet been demonstrated in the literature to our knowledge. The diagnosis of Lisfranc injuries on non-weightbearing films is a novel clinically relevant finding. Artificial intelligence requires large amounts of data which is prevalent in patient medical records. This allows for multiple machine learning models to classify, diagnose, and provide prognosis effectively due to the large amounts of patient data. Furthermore, deep convoluted neural networks can be utilized on other imaging modalities such as Ultrasound and MRI. This concept of utilizing machine learning techniques to improve diagnosis and prognosis of orthopaedic disease is a new concept with significant opportunity to improve patient care.

IMPACT OF HOXA10 ON THE PATHOBIOLOGY OF PANCREATIC DUCTAL ADENOCARCINOMA

Sophia G. Kisling, Ashu Shah, Pranita Atri, Jesse Cox, Surinder K. Batra
University of Nebraska Medical Center, Omaha, NE

Background & Significance: Pancreatic ductal adenocarcinoma (PDAC) has one of the lowest incidence rates among all major cancers, yet it is disproportionally responsible for 8% of all cancer deaths. This high death rate is primarily attributed to the immunosuppressive tumor microenvironment and a lack of clinically relevant molecular targets. In a recent study, we identified a HOXA10-driven prognostic gene signature that demonstrated a significant correlation with immunosuppressive cell types and may be combined with the TNM staging system to inform therapy interventions. The present investigation explores the functional significance of HOXA10 in PDAC, emphasizing its contribution to immune suppression and poor prognosis.

Hypothesis: We hypothesize that HOXA10 plays a crucial role in PDAC progression and promotes the immunosuppressive phenotype.

Experimental Design: *LSL-Kras$^{G12D/+}$;LSL-Trp53$^{R172H/+}$;Pdx-1-Cre* (KPC) tissues were utilized to evaluate the expression of HOXA10 throughout disease progression. Functional assessment of HOXA10 was performed using Incucyte live-cell analysis of doxycycline-inducible *Hoxa10* knockdown (KD) KPC cell lines (KPC3248). A syngeneic C57Bl/6 mouse model with orthotopically implanted inducible *Hoxa10* KD luciferase labeled KPC3248 cells was utilized to delineate the impact of Hoxa10 on tumor growth and immune milieu in the tumor microenvironment.

Results: Our analysis of KPC tissues from animals of different ages at both transcript (RNA) and protein levels demonstrated a positive relationship between HOXA10 expression and disease progression, with a trend of enhanced expression in 20 and 25-week tumors compared to 5 weeks. Live-cell analysis of *Hoxa10* KD KPC3248 cells suggested a ~2-fold decrease in their proliferation ($p<0.0001$) with concurrent enhancement of apoptosis ($p<0.0001$) as compared to the control. Interestingly, our bioluminescence measurements using IVIS imaging demonstrated a substantial delay in tumor growth in the doxycycline-treated (*Hoxa10* KD) group of mice (n=6) compared to the control group (n=4). A considerable reduction in the tumor weight was also observed at the end of the experiment. Additionally, mice harboring *Hoxa10* knockdown tumors survived longer than mice bearing the control tumors, corroborating our recent findings indicating an association between the HOXA10 signature and poor patient survival. Studies are underway to elucidate the mechanistic contribution of HOXA10 in immune suppression and poor survival.

Conclusions: These findings indicate a potential role of HOXA10 in PDAC tumorigenesis and survival. Specifically, HOXA10 may contribute to tumor burden through the enrichment of immunosuppressive phenotypes, resulting in the poor survival of PDAC patients.

TITLE: PRO-FIBROTIC EFFECTS OF MAA-MODIFIED AND/OR CITRULLINATED PROTEINS ON MACROPHAGES AND HUMAN LUNG FIBROBLASTS

AUTHORS: Lauren E. Klingemann[1], Nozima Aripova[1], Nigina Aripova[1], Michael J. Duryee[1,2], Carlos D. Hunter[1,2], Jill A. Poole[2,3], Bryant R. England[1,2], Ted R. Mikuls[1,2], Geoffrey M. Thiele[1,2]

AFFILIATIONS:

1. Department of Internal Medicine, Division of Rheumatology, University of Nebraska Medical Center, Omaha, NE
2. Veterans Affairs Nebraska-Western Iowa Health Care System, Omaha, NE
3. Department of Internal Medicine, Division of Allergy & Immunology, University of Nebraska Medical Center, Omaha, NE

BACKGROUND: Although the pathogenesis of rheumatoid arthritis-associated interstitial lung disease (RA-ILD) is not well defined, common themes such as tolerance loss, autoimmunity, and tissue fibrosis are commonly implicated in contributing to disease development. Our group and others have identified potential pathogenic roles of post-translational modifications (PTMs) such as citrullination (CIT) and malondialdehyde-acetaldehyde adducts (MAA) in RA-ILD. Both anti-citrullinated protein antibodies and anti-MAA antibodies are associated with the presence of RA-ILD. Lung tissues from patients with RA-ILD exhibited intensified staining for MAA, with pronounced co-localization with CIT. Our objective for this study was to evaluate the effects of macrophages (MΦ) activated by these PTMs on human lung fibroblasts (HLFs), focusing on pro-fibrotic responses.

SIGNIFICANCE OF PROBLEM: Clinically evident RA-ILD affects approximately 10-15% of patients with RA and accounts for the most overrepresented cause of death among people with RA with a reported median survival of only 3 years post-diagnosis.

HYPOTHESIS: Exposure of human MΦ to MAA and/or CIT fibrinogen (FIB) will lead to the secretion of soluble factors that will induce robust pro-fibrotic responses in HLFs in comparison to direct stimulation with MAA and/or CIT FIB.

EXPERIMENTAL DESIGN: HLFs were stimulated every 48 hours for 1 week either directly with FIB, FIB-MAA, FIB-CIT, or FIB-MAA-CIT or indirectly with supernatants (SN) harvested from activated U937 MΦ stimulated with the same antigens. Fluorescent immunohistochemistry (IHC) coupled with mean pixel density evaluations were utilized to monitor morphological changes and to evaluate the levels of extracellular matrix (ECM) protein deposition including collagen I, IV, VI, and vimentin. Western blots were used to validate the findings from IHC.

RESULTS/DATA: Following antigenic exposure, two phenotypically distinct morphologies were observed in HLF cells. HLF cells stimulated with MΦ-SN$^{FIB-MAA}$, MΦ-SN$^{FIB-CIT}$, and MΦ-SN$^{FIB-MAA-CIT}$ demonstrated circular and spindle-shaped changes in cellular morphology consistent with cell activation. HLF cells stimulated with MΦ-SN$^{FIB-MAA-CIT}$ showed the highest deposition of collagen I and VI compared to either single modification (*$p<0.05$) or direct antigen stimulation ($^{\Delta\Delta}p<0.01$) (Figure 1). By Western Blot, HLFs treated with MΦ-SN$^{FIB-CIT}$ and MΦ-SN$^{FIB-MAA-CIT}$ demonstrated the highest expression of collagen IV, collagen VI, and vimentin compared to MΦ-SN^{FIB} (####$p<0.001$), MΦ-SN$^{FIB-MAA}$ (****$p<0.001$) or direct antigen stimulation ($^{\Delta\Delta\Delta\Delta}p<0.001$) (Figure 2). Western blot staining for collagen I showed no reactivity (data not shown).

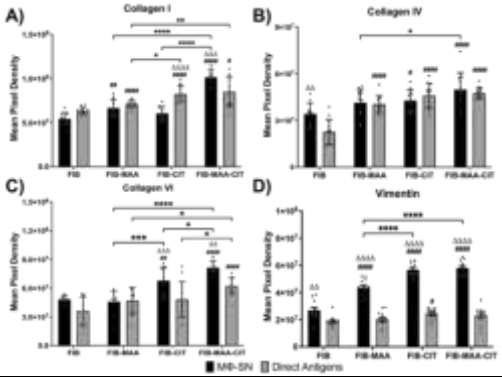

Figure 1. Quantification of Fluorescent IHC images

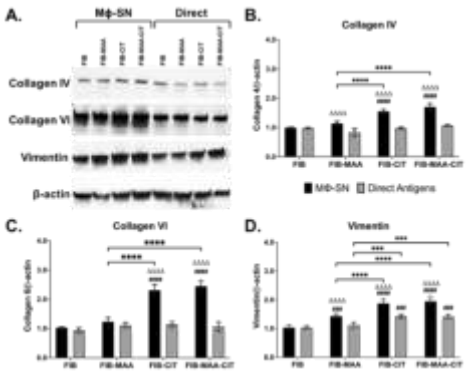

Figure 2. Western Blot Analysis of HLF cells

CONCLUSION: Findings from this study demonstrate that MΦ exposed to CIT and/or MAA modified FIB secrete soluble factors that activate pro-fibrotic responses in HLF effector cells, potentially leading to pulmonary fibrosis associated with RA-ILD. These results suggest that interventions targeting MAA and CIT formation or cell binding could represent a novel therapeutic approach in RA-ILD management.

CASE SERIES: DYSPHONIA IN CISGENDER FEMALES SECONDARY TO TESTOSTERONE TREATMENT

Violet Kryzsko, Bailey Hassman, Kayley Anderson, Patricia Foley, Kristy Carlson, Jayme R. Dowdall

University of Nebraska Medical Center, Omaha, NE

Background: Currently, there no FDA approved indication for prescription of testosterone in cisgender females. Despite this, many cisgender females are prescribed androgen-containing medications off-label for conditions such as low libido and fatigue, often without proper counseling of long-term side effects. The objective of this study is to describe cases of female patients administered testosterone with subsequent development of dysphonia.

Significance of Problem: Dysphonia and irreversible deepening of the voice for cisgender females secondary to testosterone treatment can be distressing and negatively impact the quality of life of affected patients. It is important for prescribing providers to be aware of this adverse outcome and to provide clear and comprehensive counseling to their patients prior to testosterone administration.

Problem: Cisgender females who are prescribed testosterone can experience dysphonia and permanent voice change.

Experimental Design: Retrospective chart review was conducted for cisgender female patients diagnosed with dysphonia by laryngologists at Nebraska Medicine from 2016 through 2022. Information regarding demographics, testosterone prescription, laryngoscopy evaluation, and dysphonia treatment was collected for each patient. Descriptive statistical analysis was completed.

Results. Seven patients met study inclusion criteria. All patients were Caucasian cis-gender females with an average age of 50 years old. Primary indications for testosterone administration included generalized fatigue (28.6%), hot flashes (14.2%), mood (14.2%), and (42.9%) not documented. Duration of androgen treatment ranged from over 2 years to 6 months prior to presentation for dysphonia treatment. Four patients received IM testosterone with dose ranging from 50-200 mg/ml, two patients received pellets and one patient received oral androgen with dose of 10 mg. None of the study participants received pre-dose counseling. All patients showed vocal fold edema on flexible laryngoscopy. All patients were referred to voice therapy; however, only one patient completed therapy while six patients (85.7%) were lost to follow up.

Conclusions: This case series identifies dysphonia as a significant, long-term side effect of androgen administration in cisgender females that persists despite discontinuation of medication. Our results highlight the importance of pre-dose counseling and shared decision making when choosing to prescribe these medications off-label to cisgender females, with emphasis on communicating potential risks and consideration of current evidence-based guidelines on indication, dose, and route of administration.