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P-042 IDENTIFICATION OF NOVEL TARGETS FOR THE TREATMENT OF CUTANEOUS SQUAMOUS CELL CARCINOMA
Presenter: Moynul Islam, Creighton University

P-046 EXPLORING METHYLATION OF THE CERE OF *NAEGLERIA SPECIES*
Presenter: Niklas Johnson, Creighton University

P-051 TIME FROM BIRTH TO ADMISSION FOR PRETERM INFANTS AT BERGAN MERCY
Presenter: Ryan Kimball, Creighton University

P-053 REPURPOSING THERAPIES AGAINST CISPLATIN-INDUCED HEARING LOSS
Presenter: Elise M. Kiszla, Creighton University

P-056 AGE-RELATED CELLULAR AND MOLECULAR CHANGES ASSOCIATED WITH VESTIBULAR SENSORY EPITHELIUM
Presenter: Samadhi Kulasooriya, Creighton University

P-057 ALCOHOL IN DEMENTIA: IMPLICATIONS OF ALZHEIMER'S LIKE PATHOLOGY IN ALCOHOL ABUSE
Presenter: Mohit Kumar, UNMC

P-058 hFWE DELETION REDUCED TERMINAL DIFFERENTIATION IN cSCC XENOGRAFT BUT DID NOT IMPACT TUMOR GROWTH
Presenter: Patrick Kuwong, Creighton University

P-059 DEVELOPMENT OF AN EPITHELIUM-SPECIFIC PORCINE MODEL OF PANCREATIC CANCER
Presenter: Binh Le, UNMC

P-060 THE EFFECT OF LOW-DOSAGE CORTICOSTEROID ON EARLY RENAL FUNCTION IN EXTREMELY PRETERM INFANTS
Presenter: Aron Lee, Creighton University

P-061 EFFECT OF KMT5B HAPLOINSUFFICIENCY ON CIRCULATING IGF
Presenter: Joseph Lee, Creighton University

IDENTIFICATION OF NOVEL TARGETS FOR THE TREATMENT OF CUTANEOUS SQUAMOUS CELL CARCINOMA

Moynul Islam, Justin Rudd, Louise Monga, James A. Grunkemeyer, and Laura A. Hansen
Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE

Background: Cutaneous squamous cell carcinoma (cSCC) is a form of skin cancer which affects approximately 1.8 million people annually in the US and the number of patients diagnosed with cSCC is increasing each year. The orchestrated movement of proteins and RNA between the nucleus and cytoplasm is critical for cellular homeostasis. This essential shuttling process hinges upon specialized molecular players, namely Importins, Exportins and Transportins. The irregular functioning of any of these molecular transporters has the potential to disrupt the precise localization of cargo molecules (proteins or RNA), thereby impacting cellular health. Exportins, with their pivotal role in transporting a multitude of cargoes from the nucleus to the cytoplasm, have garnered considerable scientific interest in the context of tumor development. Notably, Exportin 1 (*XPO1*) is responsible for translocating approximately 400 cargoes including tumor suppressor proteins and growth regulators. *XPO1* expression is increased in multiple cancer types. Preliminary data from our lab shows that *XPO1* is also overexpressed in actinic keratosis (pre-malignant lesion) and cutaneous squamous cell carcinoma (cSCC). Additionally, we found that Selinexor (FDA approved Exportin 1 inhibitor) is effective to cause cellular toxicity in a panel of cSCC cell lines with IC_{50} ranging from 0.071 µM to 0.74 µM and caused increased apoptosis in these cell lines. However, the challenges of limited effectiveness, and the potential for toxicity to normal cells continue to linger as substantial concerns within the realm of chemotherapeutics and Selinexor is also not an exception. The goal of this project was to identify additional targets that increase the effectiveness of Selinexor in cSCC treatment by using a genome-wide CRISPRi (inhibition) screening system.

Significance of Problem: Given the increasing incidence of cutaneous squamous cell carcinoma (cSCC) in the United States, it is important to identify highly effective chemotherapeutic options. Genome-wide CRISPRi screening holds the potential to uncover specific targets which can be used in combination with Selinexor to increase its effectiveness for cSCC treatment.

Hypothesis: Genome-wide CRISPRi screening in combination with Selinexor treatment will identify targets with potentials to increase the sensitivity of cSCC cells to Selinexor.

Experimental Design: Human cSCC SCC-13 dCas9-KRAB cells were developed for the genome-wide CRISPRi screen. SCC-13 cells were transduced with a lentiviral construct to express dCas9-KRAB, a nuclease-deficient Cas9 enzyme fused to the KRAB transcriptional inhibitor (a domain of ZNF10), along with a fluorescent reporter (mCherry). Transduced cells were sorted using FACS (Fluorescent Activated Cell Sorting) to enrich for mCherry-positive cells. SCC-13 dCas9-KRAB cells were assayed for CRISPRi inhibitory activity after transduction with a construct expressing a sgRNA targeting CD81, a cell surface marker. Flow cytometry was performed to quantify the number of cells showing CRISPRi activity for CD81 in these cells. Additionally, *XPO1* and *XPO6* (Exportin 6) were knocked down in SCC-13 dCas9-KRAB cells as another validation of the CRISPRi system. To perform genome-wide CRISPRi screen, SCC-13 dCas9-KRAB cells were transduced with lentivirus at 0.3 MOI (Multiplicity of Infection) which were packaged with Dolcetto Set A (Addgene #92385) genome-wide CRISPRi guide RNA library. The library contained 57,050 guides targeting 18,901 genes. After successful lentiviral transduction and Puromycin selection (selection marker of the library), 120,000,000 SCC-13 dCas9-KRAB were split into two groups. One group was treated with vehicle and the other group was treated with Selinexor (IC_{15}) for two weeks. Cells were sub-cultured after every 3 days by plating 60,000,000 cells per group. Genomic DNA was extracted, and PCR amplification of the guides was performed. Illumina sequencing of the PCR products was performed and the guide RNA abundance in Selinexor versus vehicle was analyzed by MAGeCK followed by gene set enrichment analysis of the underrepresented genes for identifying pathways which potentially increase sensitivity of cSCC to Selinexor.

Results: As validation of the CRISPRi efficiency of SCC-13 dCas9-KRAB cells, we observed 94% cells showing decreased expression for CD81, an approximately 90% decrease in *XPO1* and approximately 80% decrease in *XPO6* expression conferring the ability to selectively and efficiently target genes in these cells. From the genome-wide CRISPRi screen with Selinexor, 410 targets (p-value < 0.05 and log2foldchange < negative 0.58) were identified increasing sensitivity of cSCC to Selinexor. These targets are significantly enriched in pathways including telomere maintenance, RNA processing, response to DNA damage stimulus, cell cycle, regulation of biosynthetic processes, and cellular response to stress.

Conclusions: We have identified 410 targets through genome-wide CRISPRi screen that appear to increase the sensitivity of cSCC to Selinexor treatment. These targets will be further investigated for their sensitizing effect on cSCC. The identified targets may also have potential utility for combinatorial treatment in other cancer.

EXPLORING METHYLATION OF THE CERE OF *NAEGLERIA SPECIES*

Nik Johnson[1], Kristen Drescher[1]

[1]Creighton University School of Medicine Department of Medical Microbiology and Immunology, Omaha, NE

Background. The ribosomal DNA (rDNA) of *Naegleria* is encoded on a closed circular extrachromosomal elements (CERE) in the nucleolus of the trophozoite. No rDNA has been described in the nuclear DNA. It is estimated that one trophozoite contains ~4,000 copies of the CERE. The majority of the CERE sequence is comprised of non-ribosomal sequences (NRS) which is believed to contain a single origin of replication. The NRS region has little sequence conservation between species, while the rDNA cistrons are highly conserved. It is postulated that the NRS regions are involved in regulation of the ribosomal genes.

Significance of Problem. Regulation of gene transcription by the CERE of *Naegleria* is largely unexplored, but understanding how ribosome expression is regulated may provide insight into how to target treatments against *Naegleria fowleri* infections in humans, which are greater than 97% fatal. One general mechanism of gene regulation in eukaryotes is via DNA methylation. DNA methyltransferase enzymes have been described in *Naegleria*, so it appears that *Naegleria* have the means to participate in this form of gene regulation.

Hypothesis, Problem, or Question. These studies asked whether the CERE of *Naegleria gruberi, Naegleria jadini, Naegleria australiensis,* and *Naegleria pringsheimi* are methylated.

Experimental Design. Trophozoites were harvested from four species of *Naegleria* and CERE isolated. Isolated CERE were sequenced using Oxford Nanopore Technologies, the resulting data was then analyzed using dorado to determine methylation status of the cytosine.

Results/Data. Methylation was present on CERE from all species examined. Methylation was found at points across the different elements of the CERE, with little to no analogous locations of methylation across the four species investigated. The percentage of cytosines that were methylated on each CERE ranged from 0.48% - 2.8%, with *Naegleria jadini* having the lowest at 0.48%, *Naegleria gruberi* and *Naegleria australiensis* having similar levels at 1.4% and 1.6% respectively, and *Naegleria pringsheimi* having the highest level at 2.8%.

Conclusions. Methylation of the CERE of trophozoites could point towards it being a form of regulation of the rDNA. The level of methylation is on the lower end of what is seen in other eukaryotes. Eukaryotes such as the jewel wasp have 0.18% of their cytosines methylated over their entire genome, whereas gene promoters such as the promoter for HOXA11 have 30% of their cytosines methylated. However, methylation may not be the only form of gene regulation used by the *Naegleria*; other forms of regulation could include transcriptional and post-transcriptional regulation or G4-quadruplexes. The large number of predicted CERE per trophozoite coupled with the low percentage of overall methylation occurring, may indicate that there are multiple regulatory mechanisms used by the *Naegleria*. The presence of methylation in general however points towards it being involved in gene regulation. Further studies will be done to investigate methylation at the cyst stage of the *Naegleria* life cycle.

TIME FROM BIRTH TO ADMISSION FOR PRETERM INFANTS AT BERGAN MERCY

Ryan Kimball, Stevin Lu, Terence Zach
Creighton University School of Medicine, Omaha, Nebraska

Background:
Many factors may affect the time between birth and admission to the neonatal intensive care unit (NICU) for preterm infants. It is essential that the infant be in stable condition before initiating transfer. If possible, it is preferrable to give the infant some chest-to-chest time with the mother, to increase bonding and breastfeeding success. Physical distance between the delivery room and the NICU is also an important factor to consider when investigating time until admission for newborns. Lastly, gestational age at birth may affect the time until admission, which was the focus of this study. We compared the time between birth and admission to the NICU for infants of 33 and 34 weeks gestation born at CHI Health CUMC-Bergan Mercy.

Methods:
Newborn infants of 33 and 34 weeks gestation that were admitted to the NICU between January 2022 to May 2023 at CHI Health CUMC-Bergan Mercy were included. A retrospective data collection of medical records was completed to gather the following information: date of birth, time of birth, time of admission to NICU, weight, APGAR score, and gestational age. Two-sample T tests were performed to determine statistical significance, and the p-value cutoff was set at 0.05.

Results:
Our study included 114 infants: 42 born at 33 weeks and 72 born at 34 weeks gestation. The average gestational age for newborns born at 33 weeks was 33 3/7 days compared to 34 3/7 days for the newborns born at 34 weeks. The average 5-minute APGAR score for both groups was 8. The distance between the delivery room and the NICU at CHI Health CUMC-Bergan Mercy was measured to be between 150 – 300 feet, depending on which delivery room was used. The average time between birth and admission to the NICU was 22 minutes for the 33 week gestation infants compared to 23 minutes for the 34 week gestation infants ($P > 0.05$).

Conclusion:
These results suggest that there is no difference in time between delivery and admission to the NICU for infants born at 33 weeks and 34 weeks gestation. Considering that both groups had the same average 5-minute APGAR score of 8, it is understandable that the same amount of time was needed for stabilization after birth before transfer to NICU. Physical distance was likely one of the main contributing factors to time between delivery and admission to the NICU. Future research should explore if time between birth and admission to the NICU for preterm infants has an effect on other clinical outcomes, such as length of stay, growth, and development. Further, it would be interesting to compare the time until admission of infants at this institution to that of others with different layouts and explore what impact differences in physical distances makes on clinical outcomes.

Title: REPURPOSING THERAPIES AGAINST CISPLATIN-INDUCED HEARING LOSS

Authors: Elise Kiszla, Dr. Marisa Zallocchi
Creighton University School of Medicine
Omaha, NE

Background: Cisplatin, widely used to treat pediatric and adult cancer, has a high prevalence of ototoxicity, resulting in permanent hearing loss in over half of treated patients. There is a clear, demonstrated need for therapies to mitigate cisplatin-induced hearing loss (CIHL). In order to expedite the identification of potential therapies to treat CIHL, this study sought to perform high throughput screening, using a zebrafish model for cisplatin toxicity, of FDA-approved drugs currently used to treat long-term conditions.

Methods: Tg(pou4f3:GAP-GFP) zebrafish, at 5-6 days post fertilization (dpf), were randomized into 6 animals/group. For each treatment, six 5-6 dpf fish were pre-incubated for 1 hr with the drug at various concentrations between 0.5 nM to 50 µM. The fish were fixed after a 6-hour incubation with cisplatin (300-400 µM) and recovery. Neuromast hair cells were immunolabeled using otoferlin (anti-HCS-1). Three rostral neuromasts were inspected per fish by manually counting and were normalized to the control and cisplatin values to produce the relative percentage of hair cell protection. Nifedipine's effect was further classified using MitoTracker Deep Red FM, MitoTracker Red CMxROS, 500nM, and FM3-1 dyes.

Results: Atenolol at 10 uM and Nifedipine from 0.08 uM to 2 uM provided significant protection against cisplatin ($P < 0.05$). Nifedipine was chosen to be further classified. Using MitoTracker dyes, nifedipine was not shown to prevent the hyperpolarization of mitochondrial membrane potential caused by cisplatin, suggesting it provides a protective effect in another manner. Additionally, nifedipine did not prevent the uptake of FM1-43 dye, suggesting that nifedipine does not prevent cisplatin entrance into hair cells through mechanotransduction channels. Candesartan, gemfibrozil, and nicotinamide showed no significant protection against cisplatin.

Conclusions: Out of five FDA-approved drugs, atenolol, and nifedipine were the only ones to show significant protection against cisplatin-induced hair cell loss in zebrafish. They showed potential for future investigation in more relevant hair cell models (i.e., mice) to investigate their potential for clinical use to prevent cisplatin-induced hearing loss. Since Nifedipine did not significantly reduce the hyperpolarization state of mitochondrial membrane potential due to cisplatin treatment nor prevent the uptake of cisplatin through mechanotransduction channels into hair cells, further investigation should be done to determine the mechanism of nifedipine protection against cisplatin.

AGE-RELATED CELLULAR AND MOLECULAR CHANGES ASSOCIATED WITH VESTIBULAR SENSORY EPITHELIUM

Samadhi Kulasooriya, Huizhan Liu, Mi Zhou, Sarath Vijaykumar,
Celia Bloom, Litao Tao, and David Z. He
Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE

Background and significance: In adults older than 70 years, there is a 40% decrease in hair cell (HC) density in the cristae of the canals, compared to 24% in the saccule, and 21% in the utricle. In addition to HC loss, morphological and functional changes have also been demonstrated in the remaining HCs. The damaging impact of such age-related vestibular sensory decline manifests itself in an exponential increase in geriatric dizziness and a subsequent higher prevalence of injurious falls leading to Presbyastasis. While many studies have focused on age-related hearing loss and cochlear HC degeneration, age-related vestibular HC degeneration and their contribution to age-related vestibular disorders remain elusive. Hence, in the current study, we focused on our study on molecular and cellular changes associated with vestibular HC and supporting cell (SC) degeneration during physiological aging.

Hypothesis: We hypothesize that cellular and molecular changes lead to age-related degeneration of the vestibular hair cells and supporting cells.

Experimental Design: CBA/J mice aged between 2.5 and 24 months after birth were used for our experiments. We measured vestibular evoked potential (VsEP) at the system level to determine changes in vestibular function. At the cellular level, we examined changes in the morphology and ultrastructure using immunostaining (combined with confocal microscopy) and scanning electron microscopy. At the molecular level, we used single-cell RNA-sequencing (scRNA-seq) to examine changes in transcriptomes of HCs and SCs during aging. For scRNA-seq, vestibular sensory end organs of saccule, utricle, and crista were isolated. Each age group contains an equal number of males and females and a total of 100 mice were used to obtain 5 biological replicates for scRNA-seq. Droplet-based scRNA-seq was performed using the 10x Genomics chromium platform and raw data was processed by Cell Ranger to obtain count matrices. Downstream quality control, analysis, and visualization were performed in R using the Seurat package (4.3.0.1). mRNA expression will be validated by RNAscope.

Results: Our results demonstrate numerous differentially expressed genes between young and old vestibular hair cells and supporting cells indicating age-related transcriptomic changes in genes related to metabolism, ubiquitination, proteostasis, stereocilia, and cytoskeletal functions. Our gene enrichment analysis further confirmed various up- and down-regulated biological processes in response to aging. Inlining with our age-related transcriptomic changes, we observed morphological and functional changes in the vestibular sensory epithelium.

Conclusion: Overall, our findings highlight age-related alterations of the vestibule at both cellular and molecular levels. This sheds light on potential underlying mechanisms and driving factors of vestibular aging which may be conducive to developing targeted treatment strategies and delay the onset of Presbyastasis.

ALCOHOL IN DEMENTIA: IMPLICATIONS OF ALZHEIMER'S LIKE PATHOLOGY IN ALCOHOL ABUSE

Mohit Kumar, Natalie Swanson, Sudipta Ray, Viswanathan Saraswathi, Shilpa Buch*, Susmita Sil*

Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA.

Background: Alcohol use is widespread, with 84 percent of people ages 18 and older in 2021 reporting drinking alcohol in their lifetime. Mild to heavy alcohol use is associated with dysregulation at the cellular and genetic levels as well as epigenetic modifications, leading to liver injury as well as brain damage and dementia.

Significance of problem: The role of astrocytes as contributors to Alzheimer's-like pathology associated with both cognitive impairments in HIV-associated neurological disorders as well as in the comorbidity of opiate abuse, has been previously reported by our group. This underlines the importance of understanding the effects of alcohol on astrocytes.

Hypothesis: We hypothesize that alcohol could similarly induce astrocytic amyloidosis leading to neurological disorders

Experimental designs: Human primary astrocytes (HPAs) were exposed to various dose of ethanol (6.25-200 mM) for 24 hours to ascertain the dose-response effect of ethanol. Furthermore, time-dependent study was performed using a fixed ethanol concentration of 12.5 mM to monitor the cellular response over an interval extending from 0 to 96 hours. Gene silencing techniques were employed to investigate the regulatory role of lncRNA BACE1-AS. Moreover, chronic ethanol fed mice (4- weeks, *ad libitum*) were used to validate the *in vitro* findings.

Results: In this study we demonstrated, exposure of HPAs to ethanol resulted in dose dependent increase in Alzheimer's markers- amyloid precursor protein (APP), Aβ 1-42, β-site cleaving enzyme (BACE1), and inflammatory marker- IL1β as well as lncRNA BACE1AS. Next, time dependent study showed upregulation of Alzheimer's markers, oxidative stress- 4-HNE, alcohol metabolizing enzymes- alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH2), and cytochrome P450 2E1 (CYP2E1) as well as proinflammatory cytokines (TNF-α, IL1β, IL6) in the HPAs. Knockdown of lncRNA BACE1-AS in astrocytes transfected with lncRNA BACE1-AS siRNA demonstrated the regulatory role of lncRNA BACE1-AS in alcohol mediated amyloid generation, and its interaction with alcohol metabolic pathways leading to neuroinflammation and oxidative stress. Further, *in vivo* study, demonstrating up-regulation of APP, Aβ1-42, 4-HNE and IL1β in the cortex of the chronic ethanol-fed mice compared to saline.

Conclusions: This is the first report implicating the role of lncRNA BACE-AS in alcohol-mediated induction of astrocytic amyloidosis, dysregulation of alcohol metabolizing enzymes, eventually leading to neuroinflammation and oxidative stress which may contribute to cognitive impairments. These findings set the groundwork for the future development of therapeutic strategies for targeting cognitive deficits in patients with alcohol use.

Key words: Alcohol, amyloids, lncRNA BACE1-AS

hFWE DELETION REDUCED TERMINAL DIFFERENTIATION IN cSCC XENOGRAFT BUT DID NOT IMPACT TUMOR GROWTH

Patrick Kuwong, Justin Rudd, Greer Porter, Peter Halloran, James Grunkemeyer, Laura Hansen
Creighton University School of Medicine, Department of Biomedical Sciences, Omaha NE

BACKGROUND: Cutaneous squamous cell carcinoma (cSCC), is one of the most common cancers in the US and has an estimated incidence of one million cases per year. Despite being generally associated with a good prognosis, cSCCs cause significant morbidity, metastasize in approximately 4% of cases and are fatal in 2%. Epidermal keratinocytes, the cells from which cSCCs originate, form skin by organizing into stratified squamous epithelium. As these cells progress from the basal layer to the most superficial layer, they differentiate in a calcium-dependent manner.

SIGNIFICANCE OF PROBLEM: Aberrant differentiation of epidermal keratinocytes disrupts skin homeostasis and leads to multiple pathologies including cSCC, yet the mechanisms that regulate this process are not fully understood. *Flower* (*hFWE*) encodes transmembrane proteins with putative calcium channel activity but no established function in keratinocytes. Given the putative role of *hFWE* as a calcium channel and the known role of calcium in epidermal differentiation, we questioned whether **(1)** *hFWE* regulates the terminal differentiation of cancerous keratinocytes and **(2)** whether it modulates proliferative capacity and tumor growth in an *in vivo* xenograft *model*.

HYPOTHESIS: We hypothesized that CRISPR/Cas9-mediated knockout of *hFWE* in the human cSCC cell line SCC-13 would lead to impaired differentiation as well as increased tumor growth.

METHODS: To test our hypothesis, we set up a xenograft experiment in which *hFWE* wild-type (n=3) and knockout (n=3) clones of human-derived SCC-13 cells were inoculated into the subcutaneous layer of NCG immunocompromised mice (n=4 mice/group) and allowed to grow for 4 weeks. Next, mice were euthanized and tumors were collected, weighed and assessed for the expression and localization of key markers of proliferation and differentiation. CRISPR knockout. Parental SCC-13 cells were sent to Synthego (Redwood City, CA) to generate CRISPR/Cas-9 *hFWE* knockout (KO) cells. Synthego designed Cas9 sgRNA RNP sequences to knockout all 4 *hFWE* isoforms. *SCC-13 Xenograft.* SCC-13 clones ($1x10^6$ cells) were injected into the flanks of NCG mice. Tumor growth was assessed weekly by calculating tumor volume from the length and width measured via digital calipers. Tumor volume was calculated using the formula $1/2(\text{Length} \times \text{Width}^2)$. Immunofluorescence. Each tumor was formalin fixed, paraffin-embedded and immunofluorescence was performed for differentiation markers keratin 10 (SantaCruz Biotechnology- 1:200), loricrin (Biolegend- 1:200) and filaggrin (SantaCruz Biotechnology- 1:200), and a proliferation marker, PCNA (SantaCruz Biotechnology- 1:200). Automated quantification was performed using Olympus VS-ASW software. Immunoblotting. Immunoblotting was performed using antibodies for the same differentiation and proliferation markers mentioned above. Densitometric analyses was conducted using Bio-Rad's ImageLab 6.1 software (Bio-Rad Laboratories, Inc, Hercules, CA). Statistical Analysis. Statistical significance was determined using a two-tailed Student's t-test (p-values<0.05) via GraphPad Prism 10.02 software (GraphPad Software Inc., Boston, MA).

RESULTS: Quantification of filaggrin and loricrin immunofluorescence revealed a significant decrease in *hFWE* knockout xenografts compared to wild-type controls. Notably, loss of *hFWE* caused a near 60% decrease in Loricrin signal intensity, as well as a 35% decrease in Filaggrin intensity. Similar changes in the expression of these differentiation markers were observed via western blotting. However, the expression of keratin 10 remained unchanged, despite showing abnormal distribution across the suprabasal layers. Additionally, no significant differences were observed in the growth of *hFWE* knockout xenografts compared to wild-type controls four weeks post inoculation. Similarly, *hFWE* deletion had no effect on the proliferative capacity of cSCC, as shown by PCNA immunofluorescence and immunoblotting.

CONCLUSION: Together, these data indicate that loss of *hFWE* impairs terminal differentiation of cSCC, but does not affect their proliferative capacity and growth in a xenograft model.

Title: DEVELOPMENT OF AN EPITHELIUM-SPECIFIC PORCINE MODEL OF PANCREATIC CANCER

Authors: Binh Le, Carlos P Jara, Al-Murtadha Al-Gahmi, Mark A. Carlson
University of Nebraska Medical Center, Omaha, NE

Background: Five-year survival for pancreatic cancer (PC) has been ~10% for decades (currently 12%), among the worst for all cancers, which emphasizes the ongoing need for improved PC therapy. A new approach in PC research is to utilize large animal models which (i) would be useful for development of technologies that require a human-sized platform and (ii) would produce data that could have greater clinical relevance and accuracy compared to data produced by murine models. To this end, our group has been developing porcine PC models and has reliably induced PC in the LSL-KRASG12D/TP53R167H Oncopig Model (OCM).

Objective: The objective of this study was to develop a slow-growing, early-stage PC model based on the OCM by utilizing an adenoviral vector with Cre expression driven by the Keratin 8 promoter that would restrict transformation to the pancreatic ductal epithelium.

Experimental Design: We used single-cell RNA sequencing and immunohistochemistry to confirm that Keratin 8 expression in the porcine pancreas was confined to the ductal epithelium. We then confirmed that Keratin 8 promoter-driven expression of Cre recombinase (by Ad5mK8-nlsCre) was restricted to the pancreatic ductal epithelium with an ex vivo infection assay. We next performed an in vivo dose-response experiment using pancreatic intraductal injection of Ad5mK8-nlsCre (107-1010 pfu) in OCM subjects and evaluated for neoplastic changes at two months.

Results: Two months following induction of KRASG12D with Ad5mK8-nlsCre in vivo, we observed that all concentrations of adenovirus produced H&E evidence of a slowly progressing, early-stage pancreatic neoplasia in the OCM pancreas. Ductal-acinar transformation (ductal proliferation, hyperplasia with nuclear atypia, and aberrant mucin secretion) was present. Immunohistochemistry demonstrated that expression of KRASG12D was restricted to the abnormal pancreatic ducts (as confirmed with cytokeratin-7 and CAM5.2). At higher concentrations, we observed an increase in the production of mucins within the pancreatic main duct.

Conclusion: The modification to the OCM PC model generated slow-growing, early-stage ductal neoplasia, which was in contrast to the previously-observed rapidly-growing pancreatic tumors induced with nonspecific Ad5CMVCre. This slower-growing model may provide a wider therapeutic window for experimentation and testing of therapies and technologies for pancreatic cancer.

Title: THE EFFECT OF LOW-DOSAGE CORTICOSTEROID ON EARLY RENAL FUNCTION IN EXTREMELY PRETERM INFANTS

Authors: Aron Lee[1], Elizabeth Lyden[2], Eric S. Peeples[3]; Creighton University School of Medicine, Omaha, NE

Background: Preterm birth can significantly impact an infant's physical, cognitive and emotional development. One aspect of their immature physiology that exacerbates outcomes is relative adrenal insufficiency (RAI), which is defined by a lack of robust cortisol production in response to illness and stress that each infant faces. Although there are many consequences of preterm birth, one of the significant effects is immature renal structure and function. Recent studies have evaluated the potential benefits of administering postnatal prophylactic low-dose corticosteroids for the management of RAI and prevention of preterm lung disease but have not addressed their renal effects.

Significance of Problem: Steroids have been identified to hinder somatic growth, raising concerns about their impact on postnatal kidney growth and development. However, the specific effects of using low doses of steroids on kidney outcomes remain uncertain. By understanding the systemic effects of corticosteroids on RAI, our results could lead to improved outcomes for preterm infants.

Hypothesis, Problem or Question: To establish the short-term effects of corticosteroids on acute kidney function in extremely preterm infants.

Experimental Design: This retrospective study included extremely preterm infants (gestational age of 24-28 weeks) admitted to Nebraska Medical Center or Children's Nebraska between Jan 1, 2013 and Jan 1, 2023. The experimental group consisted of infants that were administered hydrocortisone within the first two weeks of life, with controls that did not receive steroids in the first two weeks. Infants diagnosed with major congenital abnormalities, transferred in after 48 hours of life, or who received any other glucocorticoids in the first two weeks were excluded. We collected pH, electrolytes, and blood pressure support for the first two weeks. Additionally, we assessed blood pressure and creatinine levels at term equivalent age and/or prior to discharge. A linear mixed model with a random effect for patient and fixed effect for day and corticosteroid groups were used for lab value trends over the first two weeks of life.

Results: When comparing demographics and early outcomes between groups, the early hydrocortisone group had more death (20.8% vs. 3.7%, p=0.026) and increased vasopressor use (70.8% vs. 29.2%, p=0.013). There were no differences in electrolytes or nutritional content over the first two weeks except for blood urea nitrogen and total daily protein administration (Fig. 1). At term equivalent age and discharge, there were no differences in creatinine, blood urea nitrogen or blood pressure between groups, although 59% of the control group received hydrocortisone after 2 weeks and 80% of controls and 67% of the early hydrocortisone group received dexamethasone after 2 weeks.

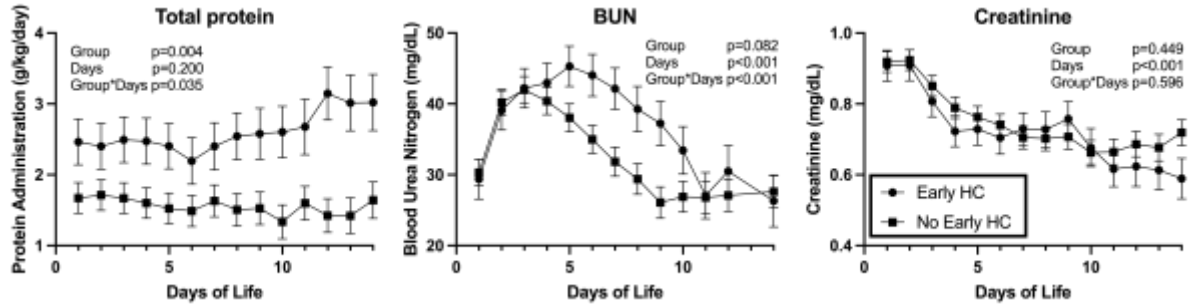

Figure 1. Total protein administration and blood urea nitrogen were higher in the early hydrocortisone group, but creatinine concentrations were similar between the two groups over the first two weeks of life

Conclusion: Our results showed no significant impact of early hydrocortisone on creatine levels and blood pressure at term equivalent age. The lack of difference at term equivalent age could have been affected by the high rate of steroid use in both groups after the first two weeks of life. Blood urea nitrogen levels were higher in the early hydrocortisone group, but this was most likely due to higher dietary protein administration rather than renal impairment. These data suggest that early hydrocortisone does not have a significant effect on short-term renal function.

Joseph Lee, Cynthia Watson, Jodi Hallgren, Holly Stessman
Creighton University School of Medicine
Omaha, NE

EFFECT OF KMT5B HAPLOINSUFFICIENCY ON CIRCULATING IGF

Background: Lysine methyltransferase 5B (KMT5B) is a gene known for its association with autism spectrum disorder (ASD). Reduced expression of this histone 4 lysine 20 dimethyltransferase results in intellectual delay and ASD in humans, as well as decreased skeletal muscle development. Despite knowing it has a role in ASD and development, the mechanism by which this occurs is not understood. Previous data from our laboratory suggest that KMT5B may regulate the expression of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 2 (IGF-2). IGF-1 and IGF-2 are both known for stimulating cell growth and proliferation and play roles in skeletal muscle and neurologic development. Growth hormone (GH) further upregulates IGF-1 production postnatally contributing to organ growth. Studying changes to IGF expression in KMT5B haploinsufficient mice (the genetic state that most closely mimics the human condition) may help elucidate the etiology of intellectual disability, ASD, and decreased skeletal muscle development in this condition.

Significance of Problem: Understanding of ASD has increased throughout society, such that more people with ASD are able to have better health outcomes and live comfortably in communities. However, more can be done to understand the pathogenesis of ASD, as well as other developmental issues that may present.

Hypothesis: KMT5B haploinsufficiency alters the expression of IGF-1 and IGF-2, leading to deficits in neurologic and skeletal muscle development.

Experimental Design: Sandwich enzyme-linked immunosorbent assays (ELISAs) were used to measure the concentrations of IGF-1 and growth hormone (GH) in mice serum over developmental time. Groups of wild type (WT; n=23) and KMT5B haploinsufficient (HET; n=24) mice, with approximately equal numbers of females and males, were terminally bled at postnatal day 17 (P17). A second group of WT (n=16) and HET (n=17) mice were serially bled each week from day P21 through P56. The weight of each mouse was measured at each blood collection time.

Results/Data: As reported previously, weights in HET mice were significantly decreased over developmental time compared to those of WT mice ($p=0.0318$-0.0016; 2-way ANOVA). IGF-1 concentration was significantly correlated with body weight in males, regardless of genotype ($p=0.0023$-0.0026; simple linear regression) with a similar yet more modest effect in females ($p= 0.0534$-0.0197). IGF-1 concentration was not correlated with GH nor GH with weight in any group. While IGF-1 concentrations were generally lower in HET compared to WT mice over time, this did not meet the level of significance ($p=0.0546$; 2-way ANOVA). However, IGF-1 was significantly lower in HET compared to WT mice at P21 ($p=0.0376$-0.0022) by Fisher's LSD post hoc testing. Alternatively, GH concentrations were significantly increased in HET compared to WT mice over time ($p= 0.0142$). This was driven primarily by increases in GH in HET mice at P17 ($p= 0.0434$; Fisher's LSD) and P28 ($p= 0.0069$).

Conclusions: KMT5B haploinsufficiency does result in decreased weight in mice but does not drastically decrease circulating IGF-1. GH, however, seems to be significantly increased in HET mice with no subsequent increase in IGF-1. This leads one to consider if there may be downregulation of IGF-1 receptors on target tissues or a negative feedback mechanism at play that should be further considered. Additionally, whether KMT5B impacts IGF-2 expression should be further elucidated as this could have effects on paracrine signaling within brain tissue.