55th Annual Midwest Student Biomedical Research Forum

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P-081 CHANGES IN TANYCYTES IN PRECLINICAL EPILEPSY: IMPLICATIONS FOR METABOLIC HOMEOSTASIS
Presenter: Parisa Rafiei, Creighton University

P-088 *FLOWER* ORCHESTRATES LATE-STAGE TERMINAL DIFFERENTIATION OF EPIDERMAL KERATINOCYTES
Presenter: Justin Rudd, Creighton University

P-089 *COXIELLA BURNETII* ACTIVELY BLOCKS INTRACELLULAR INTERLEUKIN-17 SIGNALING TO EVADE THE IMMUNE RESPONSE
Presenter: Arif Sadi, UNMC

P-090 COMPREHENSIVE ANALYSIS OF MUC16 MEDIATED AMG-510 RESISTANCE IN NON-SMALL CELL CARCINOMA
Presenter: Shamema Salam, UNMC

P-091 HEME OXYGENASE-1 INHIBITION IN PROSTATE CANCER
Presenter: Ramia Salloom, UNMC

P-093 COMPARISON OF HYPERBARIC OXYGEN TREATMENT PRESSURES FOR RADIATION-INDUCED HEMORRHAGIC CYSTITIS
Presenter: Riggs Sanchez, UNMC

P-094 GENOME ORGANIZATION IN FETAL ALCOHOL SPECTRUM DISORDERS
Presenter: Allyson Schmitz, UNMC

P-095 RAPID AUTOPSY SAMPLES ADVANCE NOVEL “CROSS-XENOGRAFT” ULTRASOUND GUIDED INJECTION MODEL
Presenter: Caroline Seilstad, UNMC

P-096 DESIGN, SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIP STUDIES OF BENZAMIDE-BENZIMIDAZOLE ANALOGUES AS QUORUM SENSING INHIBITORS IN *PSEUDOMONAS AERUGINOSA*
Presenter: Mishra Shreeya, Creighton University

P-097 BIOACTIVE METABOLITES OF POLYUNSATURATED FATTY ACIDS IN THE PLACENTA ARE ASSOCIATED WITH PREGNANCY OUTCOMES
Presenter: Rebecca Slotkowski, UNMC

CHANGES IN TANYCYTES IN PRECLINICAL EPILEPSY: IMPLICATIONS FOR METABOLIC HOMESOTASIS

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Background: Epilepsy neurological disorder that causes spontaneous recurrent seizures (SRS) due to sudden surges of abnormal and excessive electrical activity in the brain. Seizures can induce injury, ectopic neurogenesis, and metabolic dysfunction. The hypothalamus has regulatory influence over important functions including sleeping, cardiorespiration, emotion and eating. Tanycytes are cells in the hypothalamus that surround the third ventricle and facilitate communication between the CSF and local hypothalamic regions to ensure metabolic homeostasis. One of the therapeutic treatments which is effective in reducing seizure frequency is the Ketogenic Diet (KD). The ketogenic diet is a high fat, low carbohydrate/protein diet. The ketogenic diet reduces seizure frequency by >50% in approximately two thirds of patients with refractory epilepsy.
Significance of Problem: It is unknown whether tanycytes are dysregulated by seizures or not. Considering that severe seizures can propagate down to the hypothalamus, it is important to understand how spontaneous recurrent seizures influence tanycytes due to their vital role in metabolic homeostasis.
Hypothesis: Seizures dysregulate tanycytes in preclinical epilepsy.
Experimental Design: We conducted experiments by using *Kcna1*-null mice, which is a preclinical model of spontaneous recurrent seizures. *Kcna1* gene provides instructions for making one part (the α subunit) of a potassium channel called Kv1.1. Expression of $K_V1.1$ α subunit results in delayed rectified potassium current. This specific mouse model has a deletion mutation of their *Kcna1* gene which results in the $K_V1.1$ Knockout mice. We used fluorescent immunohistochemistry and random systematic stereology to determine changes along the dorsal-ventral aspect (alpha and beta tanycytes, respectively) and along the anterior-posterior aspect of the third ventricle. Furthermore, we administered ketogenic diet treatment for our mouse model as the treatment group for a duration of 2 weeks.
Results: We found elevated levels of (i) GFAP, a marker for all tanycytes (p <0.05) along the third ventricle and reduction of (ii), GLUT1, a functional marker of glucose transport (p <0.01) in the medial-posterior aspects of the thirds ventricle. Additionally, we found that the ketogenic diet had no effect in the expression of either GFAP or GLUT1.
Conclusions: 1) Even though previous studies have shown that KD is effective against seizures, here, it did not influence tanycytic protein levels in this preclinical epileptic mouse model. **2)** The significant elevation in GFAP expression in tanycytes may be similar to the GFAP increase observed in astrogliosis associated with seizures, and thus pathologic. **3)** The reduction in GLUT1 in this model of SRS may impact metabolic homeostasis in the local hypothalamic nuclei. **4)** It is important to note that despite the KD not influencing GLUT1 expression in this SRS mouse model, it remains an important option as a treatment for specific epilepsy types as well as neurodegenerative diseases.

FLOWER ORCHESTRATES LATE-STAGE TERMINAL DIFFERENTIATION OF EPIDERMAL KERATINOCYTES

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Defects in epidermal differentiation compromise its barrier function and lead to numerous skin diseases including ichthyosiform and acantholytic disorders, as well as skin cancer, which together impact more than 20% of the world's population. Improved understanding of homeostatic epidermal differentiation promises to uncover new therapeutic targets for these and the numerous other diseases that affect epidermal barrier function. Calcium plays a critical role in differentiation of the epidermis, which establishes a calcium gradient, with the highest concentrations in the stratum granulosum (SG). SG keratinocytes form tight junctions (TJs) and secretes lamellar body (LB) contents at their apical surface before undergoing corneoptosis. The mechanisms by which calcium is elevated in the SG and the relationship between calcium and the dramatic polarization of intracellular contents in the SG remain unclear. We **hypothesized** that the Flower protein (hFWE4) plays a role in executing the molecular events that are essential for polarized secretion of LBs during these final stages of epidermal differentiation in the SG. Our recently published work demonstrated that hFWE4 localizes to intracellular vesicles carrying unknown cargo to the plasma membrane in epithelial cells, but a role for hFWE4 in skin has never been described. Here **our results** show that in skin, hFWE4 localizes to apically polarized cytoplasmic vesicles in the SG that fuse wait the interface of the stratum corneum (SC). In organotypic epidermis culture, *FWE* knockout keratinocytes show defective terminal differentiation and barrier function. Proteomic data (CoIP-MS, and LFQ surface proteomics) demonstrate that hFWE4 binds to calcium-regulated vesicular adaptor proteins, exocyst complex subunits, and SNARE proteins in keratinocytes, and overexpression increases the surface presentation of numerous LB and TJ proteins. Live cell super-resolution confocal and surface biotinylation-immunoblotting experiments were used to validate direct trafficking of a multi-functional LB cargo molecule, TROP-2. Together, these data suggest a functional role for hFWE4 in trafficking of LB to the cell surface for exocytosis. Additionally, GCaMP-based calcium imaging experiments also demonstrate that hFWE4 overexpression potentiates the cytosolic calcium elevation in response to thapsigargin and extracellular calcium addition, suggesting that it may have direct calcium channel activity. Lastly, using immunofluorescence of clinical specimen and analysis of publicly available patient RNA-seq data, we show that hFWE expression and subcellular localization are dysregulated in Grover's and Darier disease, acantholytic disorders that are driven by impaired cytosolic Ca^{2+} handling. From these and other data, **we conclude** that hFWE4 acts as a critical regulator of epidermal morphogenesis by facilitating calcium-dependent, apically polarized trafficking of LBs in SG keratinocytes. Future directions will define mechanisms through which hFWE4 mediates the release of Ca^{2+} from vesicular stores to recruit trafficking machinery to LBs during epidermal barrier formation, and delineate how dysregulation of these mechanisms contributes to acantholytic skin disease.

Coxiella burnetii ACTIVELY BLOCKS INTRACELLULAR INTERLEUKIN-17 SIGNALING TO EVADE THE IMMUNE RESPONSE.

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Coxiella burnetii is an obligatory intracellular bacterium and the causative agent of Q fever. The *C. burnetii* type IVB secretion system (T4BSS), which translocates bacterial effector proteins over the pathogen-containing vacuole membrane and into the host cytoplasm, is necessary for successful host cell infection. In host cytoplasm, they influence various cellular processes, and one of the main signaling pathways the *C. burnetii* T4BSS manipulates is the interleukin-17 (IL-17) pathway. IL-17A, mainly produced by a distinctive subgroup of CD4+ T cells named Th17, manages local tissue inflammation by releasing proinflammatory cytokines. Furthermore, IL-17 plays a crucial role in activating chemokines and neutrophil migration. Although IL-17 is involved in the pathogenesis of several autoimmune diseases such as rheumatoid arthritis, psoriasis, and asthma, this cytokine also acts as an essential factor in host defense against microbial infections in the lung. One of the vital components in IL-17-mediated signaling is the NFκB activator 1 (Act1), which recruits Tumor necrosis factor-R-associated factor 6 (TRAF6), an essential upstream activator of the NF-κB pathway. Although earlier research has shown that IL-17 is protective against various infections, it is unclear what function IL-17 serves during *C.burnetii* infection. We hypothesize that *C. burnetii* T4BSS downregulates intracellular IL-17 signaling to elude the host immune response and advance bacterial pathogenesis. The inhibition of IL-17 transcriptional activation by *C. burnetii* T4BSS was verified employing a stable IL-17 promoter reporter cell line. Furthermore, we observed that *C. burnetii* T4BSS suppresses the macrophage proteins CXCL2/(MIP)-2 levels and CCL5/RANTES following IL-17 stimulation. Further, using IL-17RA and TRAF6 knockout cells, we show that *C. burnetii* can block IL-17-mediated neutrophil migration. In addition, *C. burnetii* T4SS effector proteins inhibit oxidative stress induced by IL-17, indicating that *C. burnetii* inhibits IL-17 signaling to evade macrophage-driven killing. All these findings suggest that *C. burnetii* suppresses IL-17 signaling in a T4BSS-dependent manner to evade the immune response.

COMPREHENSIVE ANALYSIS OF MUC16 MEDIATED AMG-510 RESISTANCE IN NON-SMALL CELL CARCINOMA

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Background

Lung cancer is the leading cause of cancer death in the US, accounting for about 1 in 5 of all cancer deaths. Chemoresistance results in tumor recurrence and is a frequent cause of cancer-related death in non-small cell lung cancer (NSLC). MUC16 overexpression is associated with tumorigenesis, metastasis, poor survival, and reduced sensitivity to existing therapies. The most common KRAS mutation in NSLC, KRASG12C (13%), causes tumor initiation and progression. Despite the availability of several approved KRASG12C inhibitors {sotorasib (AMG510) and adagrasib (MRTX849)}, the overall five-year survival rate remains poor (25%) as most patients often develop resistance towards these drugs. Preliminary studies from our lab indicate an enhanced expression of MUC16 in KRASi (AMG510)-resistant NSCLC cells and, therefore, suggest its involvement in therapy resistance. Our lab has recently developed a novel anti-MUC16 chimeric monoclonal antibody, mAb5E6 (ch5E6), which exhibits anti-proliferative effects alone as well as in combination with AMG510 in tumor xenografts of NSCLC.

Hypothesis/Rationale

This study aims to delineate the underlying mechanisms of AMG-510 therapy resistance and evaluate the efficacy of MUC16 targeting by chimeric mAb5E6 in overcoming therapy resistance in NSCLC.

Experimental Design

We performed MUC16-associated gene and pathway analysis on RNA seq database (GSE137912) of AMG-510 resistant G12C mutant (72 hrs.) SW1573, H2122, and H358 cell lines. We then evaluated the efficacy of chimeric mAb5E6 in combination with AMG-510 in a panel of G12C mutant NSCLC cell lines. This was followed by assessing marker genes associated with proliferation, apoptosis, and AMG-510 resistance.

Results

We identified common genes, including RPS4X, SAT1, NEAT1, GNB2L1, EEF2, and IFITM3, associated with MUC16-mediated AMG-510 resistance across a panel of cell lines. These genes are being validated in AMG-510-resistant tumors from cell line-derived xenografts and NSCLC patients. Treatment of SW1573 cells with ch5E6 in combination with AMG-510 resulted in a substantial decrease in their proliferation. Moreover, IHC analysis of ch5E6 treated tumors demonstrated a significant decrease in percentage of Ki67 (44%) cells with a concomitant increase in apoptosis % cleaved caspase 3(23%) than isotype control treated group. Furthermore, a synergistic effect was observed in tumors treated with ch5E6+ AMG-510 combination regimen (Ki67= 25%, cleaved caspase 3=17.6%) than in AMG-510 treatment (Ki67= 66%, cleaved caspase 3=12.3%) indicating the potential of ch5E6 in overcoming AMG-510 resistance.

Conclusions

Our preliminary results identify the key genes associated with MUC16-mediated AMG-510 resistance. Furthermore, chimeric mAb5E6 enhanced the anti-proliferative activity of MAG-510-resistant NSCLC cells. In the future, this study will extensively delineate the mechanism of AMG-510 resistance and utilize anti-MUC16 chimeric mAb5E6 to overcome therapy resistance in NSCLC.

HEME OXYGENASE-1 INHIBITION IN PROSTATE CANCER

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Abstract:

Prostate cancer (PC) remains a global health challenge, with an estimated 288,300 new cases in 2023, necessitating innovative approaches to enhance therapeutic efficacy. Chemotherapy is the primary treatment for castration-resistant prostate cancer (CRPC), and Docetaxel (Doc) is one of the most effective chemotherapeutic agents for CRPC. However, unfavorable side effects and chemoresistance reduce the efficacy of Doc. Hypoxia in PC is correlated with chemoresistance to Doc-induced apoptosis and reprograms the expression of Heme Oxygenase-1 (HO-1), the inducible form of Heme Oxygenase.

HO-1, the enzyme that controls the initial and rate-limiting step in heme degradation, is induced by oxidative stress and produces free iron (Fe), along with the anti-inflammatory product biliverdin and carbon monoxide, which promote cell proliferation and survival against oxidative stress caused by Doc. This study focuses on HO-1 inhibition as a strategy to potentiate the therapeutic effect of Doc and explores the potential of combining its administration with pharmacological HO-1 inhibitors.

In this study, diverse PC cell lines were utilized in vitro to assess the impact of HO-1 inhibition, alone and in combination with Doc, on different PC cells parameters. Our findings demonstrate that HO-1 inhibition significantly reduces PC cell viability under hypoxic and normoxic conditions. Also, our treatment sensitizes PC cells to Doc-induced apoptosis through a series of interconnected mechanisms. These mechanisms include increased expression of reactive oxygen species (ROS), disruption of the glutathione cycle, and alteration in the STAT1 (Signal transducer and Activator of Transcription 1) pathway. This observation implies an interplay between STAT1 and HO-1, suggesting that STAT1 works through HO-1 activation. These collective findings suggests that HO-1 inhibition might be a key factor in sensitizing PC cells to Doc and potentially addressing the challenge of acquired resistance.

These results propose a promising approach of combining HO-1 inhibition with Doc to enhance therapeutic outcomes in PC. By uncovering the intricate interplay between HO-1 inhibition and the apoptotic pathway, shedding light on this cooperative effect, our research advances the field of targeted treatment strategies, with the potential to enhance clinical management and ultimately improve patient outcomes in prostate cancer therapy.

COMPARISON OF HYPERBARIC OXYGEN TREATMENT PRESSURES FOR RADIATION-INDUCED HEMORRHAGIC CYSTITIS

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Rates of kidney, bladder, and prostate cancers have increased worldwide and have presented an increasingly significant health burden.[1] The current treatment includes radiotherapy (RT), which targets and kills cancer cells with ionizing radiation and may result in damaging side effects, including ulceration, decreased tissue regeneration, and fibrosis of the bladder wall.[1] Approximately 3-6.5% of these patients develop radiation-induced hemorrhagic cystitis (RIHC) with symptoms presenting from 6 months to 20 years post-radiation treatment.[2] The complications of RIHC result in 7% of emergency urology admissions, leading to significant health burdens to patients and hospital systems.[3] Treatment for routine cases involves the management of symptoms, but refractory cases require urinary diversion and cystectomy, which carries a 44% mortality rate.[3] For non-emergent RIHC, hyperbaric oxygen therapy (HBOT) is a recommended treatment. The higher pressure of oxygen promotes tissue healing and angiogenesis, which is accomplished by creating a steep oxygen gradient between capillaries and tissues.[4] Response rates of HBOT in patients with RIHC have reported rates from 80%-100%.[2] Despite high success, there is no definitive knowledge of the ideal treatment pressure, duration, or number of treatments. This project aimed to determine the difference in outcomes for patients treated at 2.4 and 2.0 atm. A retrospective chart review was performed of 53 patients who received HBOT at Nebraska Medicine between 2014 and 2023. During the review, data was collected on cancer history, treatment protocols, medical history, and basic demographics. Success was graded based on changes in symptoms of cystitis and placed into three categories: worsening symptoms, little to no change in symptoms, and improvement of symptoms. Improvement of symptoms for patients treated at either 2.4 atm or 2.0 atm were comparable with rates of 93.5% and 86.4%, respectively. However, the difference between the two treatments was not statistically significant. Overall, 90.5% of patients had clinical improvement greater than the average values reported in two systemic review articles. Of the cohort, five patients (9.4%) had no improvement, and 17 patients (32.1%) had a clinical recurrence of symptoms prompting alternative treatments. The study found that the methods of HBOT showed success rates similar to those published in the literature and that the current methods are effective at treating RIHC. No significant comorbidities were identified, and no temporal variables were found to be significant. The study found that there was no statistical difference in outcomes for patients treated at different pressures, although there was a trend towards the higher pressure.

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GENOME ORGANIZATION IN FETAL ALCOHOL SPECTRUM DISORDERS

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Background:

Fetal alcohol spectrum disorders (FASD) are a spectrum of disorders that are characterized by cognitive or behavioral deficits, abnormal facial features, growth retardation, and occasionally structural birth defects. Prenatal alcohol exposure has not shown to cause direct mutagenic effects on the genome, therefore much work has been focused on potential epigenetic alterations induced by fetal alcohol exposure. CTCF loops are loops of DNA formed when cohesin protein extrudes DNA until it encounters DNA- bound CTCF, which forms the boundaries of these loops. These loops facilitate long-range DNA:DNA interactions, such as between enhancers and promoters, and so may modulate gene transcription. Our goal in this study was to characterize the effects of disrupted genome organization induced by ethanol through altered CTCF binding, and ultimately what effects this has on gene transcription

Significance of Problem:

FASD has detrimental effects on the developing child, such as delayed motor and speech development, decreased cognitive abilities, behavioral issues and impaired interpersonal skills. This study provided information about the epigenetic mechanisms contributing to the etiology of FASD, and which may be utilized going forward in the development of treatments aimed at decreasing the severity of FASD.

Hypothesis:

Ethanol produces changes in genomic architecture and transcription of local genes, leading to abnormal signaling pathways important in the pathogenesis of fetal alcohol spectrum disorders.

Experimental Design:

HCT116 cells were treated with ethanol at concentrations of 30, 90, or 200 mg/dL for six days. After treatment, ChIP-seq was performed to identify DNA binding sites for CTCF and cohesin, and ATAC-seq (Assay for transposase-accessible chromatin) was used to identify regions of open chromatin in the genome. Data analysis from ChIP-Seq and ATAC-seq was performed through in-house bioinformatic pipelines.

Results:

On ChIP-seq analysis, broad disruption of CTCF localization on chromatin was demonstrated, including sites with gained CTCF binding and sites with lost or decreased CTCF binding. A dose-dependent effect from ethanol was seen in gained CTCF peaks, however lost CTCF peaks did not show a dose-dependent response. ATAC- seq similarly demonstrated differential areas of open and closed chromatin between treated and untreated samples.

Conclusions:

This project provided essential preliminary data for additional studies to more completely characterize the epigenetic changes in FASD. After establishing differential CTCF occupancy on chromatin, techniques such as Hi-C can be used in future studies to analyze genome-wide chromatin:chromatin contacts and more directly assess genomic architectural changes induced by ethanol. This work will build toward a greater understanding of the molecular alterations induced by ethanol, with the ultimate goal of identifying intervenable pathways for potential therapeutics.

RAPID AUTOPSY SAMPLES ADVANCE NOVEL “CROSS-XENOGRAFT” ULTRASOUND GUIDED INJECTION MODEL

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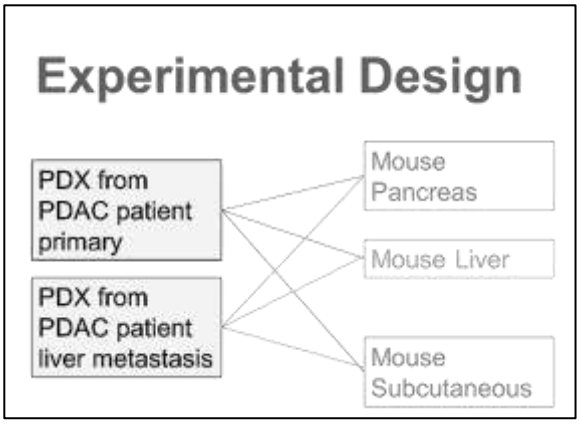
Background: Mimicking the human condition is a major challenge in biomedical research today. We previously developed a unique model of studying disease: human samples from our Rapid Autopsy Program (RAP) used to develop xenografts (PDX) from matched sets of primary tumor and different metastatic sites. This model enables direct comparison of PDX material to the original patient samples for characterization of molecular and biological properties. We employ subcutaneous, under the skin, orthotopic, matched organ, or ectopic, unmatched organ, sites of surgical implantation for study purposes.

Significance of Problem: Orthotopic xenograft models allow the growing tumor to receive and send signals in a similar environment, whereas subcutaneous and ectopic sites allow for comparison to different environments. Typical implantation requires physically traumatic open surgery with a long recovery period and costly longitudinal studies to image changing tumors. In current orthotopic and ectopic liver models, potential blood loss increases the mortality risk, scarring from the large surgical opening distorts further imaging, and splenectomies greatly impact the immune environment.

Hypothesis: We propose a comprehensive minimally invasive model to decrease the stress of orthotopic surgery that will allow us to distinguish microenvironment and cellular dependent changes.

Experimental Design: We will optimize an ultrasound guided injection (USGI) technique to implant cells into a target organ without opening the animal. This USGI model minimizes recovery time, from days to minutes, and eliminates inflammation related to surgical intervention. In order to compare the genomic and transcriptomic changes that occur between surgical models and the human samples, we developed this ‘cross-xenograft’ technique to inject paired pancreas primary and liver metastasis cell lines and dissociated tumors from the RAP biobank, into orthotopic and ectopic locations. Using a handheld ultrasound, we will further increase reproducibility by improving accessibility to this technique as well as longitudinal studies.



Results/data: We achieved successful injection of RAP derived cell lines at an 80% take rate. Reflecting surgically implanted orthotopic tumors directly taken from patients, we saw a take rate of 14% in RAP derived dissociated tumor injection. With a handheld ultrasound, we were effective at injecting pancreas, liver, renal sub capsule, and spleen and found the ease of access and user friendly interface instrumental in longitudinal imaging studies. Contrary to previous surgical models, no animals were lost to hemorrhage, scars did not obstruct further imaging, and immune function remained intact.

Conclusions: Data derived from these samples will allow us to assess tumor-environment effects on clonal selection and evolution, and to characterize differences between the original human sample and corresponding animal PDX model. This cutting-edge USGI “cross-xenograft” model eliminates variables associated with traumatic surgery. Overall, this model system will be utilized to clarify strengths and limitations of xenograft models and should provide better insight into how to accurately incorporate orthotopic and ectopic models of tumor growth into biomedical research.

DESIGN, SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIP STUDIES OF BENZAMIDE-BENZIMIDAZOLE ANALOGUES AS QUORUM SENSING INHIBITORS IN *PSEUDOMONAS AERUGINOSA*

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The emergence of multidrug-resistant (MDR) strains of *Pseudomonas aeruginosa (PA)* becomes a public health threat; indeed, in 2017, World Health Organization recognizes resistant *PA* in the "critical" group (WHO 2017). *PA* is a Gram-negative bacterium that causes opportunistic infections in immunocompromised patients. *PA* has ability to develop intrinsic and adaptive resistance including inducible AmpC and OXA enzyme expression, efflux pump production (constitutive and inducible) and low outer membrane permeability (Horcajada et al. 2019). Pseudomonas recognizes its population by releasing autoinducer chemicals such as Pseudomonas Quinolone Signal (PQS) and 4-hydroxy-2-heptylquinoline (HHQ) via quorum sensing (QS) systems. PQS and HHQ both activate the transcriptional regulator Multiple Virulence Factor Regulator (MvfR), which regulates QS molecule synthesis, toxins, and biofilm formation. A biofilm decreases antibiotic delivery and efficacy in cells, rendering them ineffective. Thus, MvfR that is pivotal for the development of acute infections and antibiotic-tolerant, has emerged as an essential target for the pharmacological interventions by small molecule inhibitors. Ongoing basic efforts to tailor MvfR activity have identified 1st and 2nd generation Benzamide-nitrobenzimidazole inhibitors as antibacterial agents *in-vitro* and in infection mice models. Problems such as, less potency (have sub-micromole inhibition), severely less solubility, mutagenicity (possibly due to problematic aromatic nitro-group) and low exposure (indicate that drug is quickly cleared out of the animal's system, leads to immuno-genicity) refrain these inhibitors from being progressed to lead molecules. The absolute requirement of the MvfR for development of antibiotic resistant virulence in pathogens, and the fact that it has no host cell counterpart, still makes it an attractive drug development target however, more potent and specific inhibitor with better pharmacokinetic (PK) are prerequisite to achieve fully the therapeutic efficacy of this target. Thus, chemical modulation to extensive structure activity relationship (SAR) studies are must to achieve desired potency and inhibitors with better PK profile. In this proposal, we intend to address above mentioned problems and accommodate desired characteristics to develop novel small pharmacophores for the accomplishment of a paradigm shift in antimicrobial therapy.

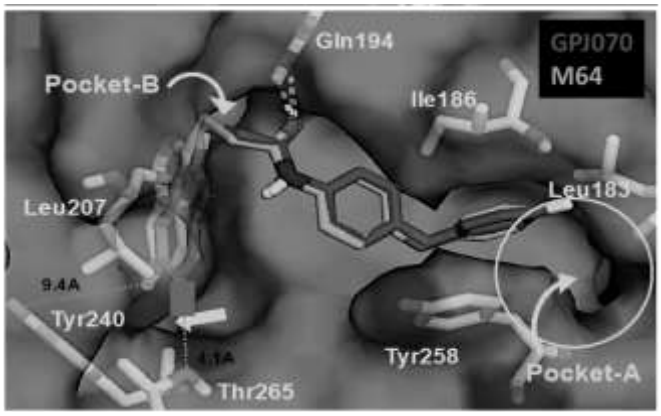

Figure 1: Overlay showing docking poses of GPJ070 (magenta) and M64 (cyan) into the binding pocket of MvfR surface view structure. Compound with *p*-fluoro substitution on phenoxy ring (magenta) forms additional H-bonds with Leu183; deep unexplored pockets (yellow arrow and circle). (PDB: 6B8A was employed for molecular docking (Schrodinger software) and figures were generated using Pymol).

Our preliminary studies employed an overlay of recently reported crystal structures of MvfR agonist-HHQ (PDB:4JVD) and antagonist-M64 (PDB: 6B8A) to obtain ligand-MvfR binding coordinates within the binding pockets essential for the bacterial inhibitory mechanism. These coordinates also revealed that M64-antagonist lack enough structural features to interact with putative binding residues of hydrophobic region of MvfR (Fig 1, Pocket-A) and could be modified chemically for better potency and efficacy. M64 has solubility issue and indicated mutagenicity in ames-II test. Molecular docking of M64-MvfR further revealed that chemical modulation of M64 structure at its Phenoxy-phenyl ring could develop less toxic and more water soluble analogs. Thus, we constitute our *central hypothesis* that molecular docking develops novel MvfR inhibitors with a set of excellent quality for preclinical.

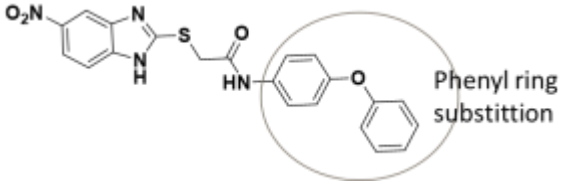

Figure 2: Structure activity relationship of M64 molecule on Phenoxyphenyl ring (shown as orange circle)

Total of 18 compounds were synthesized by modulating phenoxyphenyl part of M64 (Fig 2, orange cirle). These compounds were assessed by a high throughput screening (HTS) assay for repression of pyocyanin (PYO) production at 1 µM in triplicate in a PA14 (*Pa* strain UCBPP-PA14 which produces 10 times more PYO than strain PAO1 was used). GPJ-compounds and DMSO (control) for which individual replicates showed inhibitory activity that differed by three standard deviations were used for IC_{50} determinations. GPJ compounds inhibit PYO in a dose dependent manner with equi or more potent IC_{50}s, than M64. Phenoxy group shift to *meta* position and its replacement by phenyl, 2-Me-phenyl, and electron rich triazolyl-methyl retains inhibition. Electron deficient *o*-chloro and substitution on phenoxy increases inhibition by 7.5-fold (GPJ073). GPJ-073 is nontoxic and retains more than 90% cell viability of human U937 macrophages in MTT assay.

BIOACTIVE METABOLITES OF POLYUNSATURATED FATTY ACIDS IN THE PLACENTA ARE ASSOCIATED WITH PREGNANCY OUTCOMES

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Background: Inflammation plays a key role in healthy fetal development during pregnancy. However, excessive inflammation in the placenta is associated with severe maternal and infant morbidities such as pre-eclampsia, preterm birth, or poor infant growth trajectories. Polyunsaturated fatty acids (PUFAs) and their bioactive metabolites have been shown to regulate inflammation, but little is known about how PUFA metabolites in the placenta may affect pregnancy outcomes.

Significance of Problem: Placental inflammation can have life-threatening consequences for mom and baby. PUFA metabolism in the placenta may mitigate excessive inflammation to prevent subsequent adverse pregnancy outcomes.

Hypothesis: We hypothesize that placental PUFA metabolite concentrations will be higher among maternal-infant dyads who experienced pregnancy complications, compared to those with a healthy pregnancy.

Experimental Design: Basal (maternal) and chorionic (fetal) placenta sections were analyzed for bioactive PUFA metabolites using liquid-chromatography-tandem mass spectrometry. Pregnancy outcomes (pre-eclampsia, delivery mode, and low birth weight) were collected from medical records. Mann-Whitney U tests were used to compare placental PUFA metabolite concentrations across pregnancy outcomes.

Results: The median maternal age was 28.5 years (IQR: 23.0 – 33.3) and the median infant gestational age was 39.0 weeks (IQR: 36.4 – 39.5). Maternal-infant dyads with pre-eclampsia (n=13) had significantly higher basal and chorionic placenta concentrations of multiple PUFA metabolites derived from multiple enzymatic pathways compared to dyads without pre-eclampsia (n=51). Similarly, dyads who experienced a caesarean section (n=15) had significantly higher placental concentrations of multiple PUFA metabolites compared to dyads who delivered vaginally (n=50). Placenta metabolite concentrations were also significantly higher among low-birth-weight infants (n=11) compared to infants with a birth weight >2500 grams (n=54).

Conclusion: Our study consistently found higher PUFA metabolite concentrations in the placenta from maternal-infant dyads who experienced pregnancy complications compared to dyads with a healthy pregnancy. Our findings may indicate that PUFA metabolism is upregulated in the placenta during inflammation, either as a dysfunctional result of excessive inflammation or an adaptive mechanism to mitigate the effects of inflammation.