Abstracts 2023

RUTGERS HEALTH
New Jersey Medical School

RUTGERS HEALTH
Robert Wood Johnson Medical School

RUTGERS HEALTH
School of Dental Medicine

RUTGERS HEALTH
School of Health Professions

NIH R25 Multidisciplinary Opportunities in Research Education for Students in Health Professions

Valerie A. Fitzhugh, MD, Diego Fraidenraich, PhD, Pranela Rameshwar, PhD

Program Directors

Expression of Bone Morphogenic Protein 10 (BMP10) in T cells responding to Endogenous Retroviral Super Antigen vSAg29

Student: Oluwadamilola Adetayo

Mentor: Vincent Tsiagbe, PhD

Bone morphogenic protein 10 belongs to a family of about 20 BMPs in humans. BMPs members of the transforming growth factor-β (TGF-β) superfamily. The BMPs have important roles in development and organogenesis by delivering positional information in both vertebrates and invertebrates. BMP10 has been shown to be involved in cardiac hypertrophy. Further recent study has demonstrated the expression of BMP10 in lymphocytes in a rat model for periodontal disease, induced by *Aggregatibacter actinomycetemcomitans (AA)* bacteria. The expression of BMP10 in lymphocytes was demonstrated to increase during the development of bone resorption. Such an increase begged the question that BMP10 might be involved in activation of lymphocytes responding to antigens. The involvement of retroviral superantigen (vSAg29) in the development of an aggressive B cell lymphoma in a mouse model for human germinal center-derived B cell lymphomas, prompted an examination of the role for BMP10 in the "reverse immune surveillance" mechanism that drives robust CD4 T cell response which provides growth factors for lymphoma development. The purpose of this study is to examine BMP10 expression in CD4+ T cells responding to vSAg29+ B cell lymphoma. This study provides preliminary evidence that retroviral antigen (vSA29) activated CD4 T cells enhance the expression of BMP10, in a manner resembling the bacterial antigen activation of lymphocytes in the development of periodontal disease. The mechanisms mediating the upregulation of BMP10 remains to be determined by further research.

A New Role for Connexin-43 in Duchenne Muscular Dystrophy Associated Cardiomyopathy

Student: Ifeanyichukwu Adibemma

Mentor: Diego Fraidenraich, PhD

Introduction: Duchenne Muscular Dystrophy (DMD) is a progressive neuromuscular disorder caused by an X-linked recessive mutation that results in the absence of dystrophin protein.

Dystrophin is an essential scaffold protein that serves as a link between the cytoskeleton of myocytes and extracellular matrix. A defect in this protein results in a compromised structural integrity of muscles cells, primarily in the heart and skeletal muscle. Clinically, the disease manifested through muscle degeneration, chronic inflammation, and impaired motor function. Although there is no definitive cure for DMD, current treatment options including corticosteroid and physical therapy, are used to manage symptoms and improve quality of life. Strides have been made in recent decades to improve standard treatment plans for DMD patients, yet this population has a notable decrease in life expectancy. As of 2021, the median life expectancy for a DMD patient is 28.1 years (1).

Arrhythmia resulting from cardiomyopathy is the most common cause of death in patients with DMD. In addition to impaired structural integrity and resiliency to mechanical stress, absence of the dystrophin protein in the heart also impacts electrical conduction and signaling pathways that are essential for normal cardiac function. In particular, a gap junction protein called connexin-43 has been implicated to play a vital role in intercellular communication across cardiomyocytes.

These hemi-desmosomes transport ions such as sodium, potassium, calcium and ATP, contributing to synchronous electrical propagation of cardiomyocytes at the intercalated disks. Defects in dystrophin synthesis contribute to connexin-43 remodeling, a process which describes alteration to normal cardiac tissue. Specifically, absence of dystrophin protein appears to trigger a series of events which ultimately increases lateralization of connexin-43, increase connexin-43 density, decrease phosphorylation of connexin and increase microtubule density.

Furthering our understanding of the effects of DMD on cardiac tissue, several studies have highlighted a relationship between phosphorylation of connexin-43 and remodeling. Using gene editing technology, a serine amino acid in the serine amino acids at positions 325,328, and 330 of Cx43 protein is replaced with a glutamic acid group, serving as a phospho-mimic. The addition of the phospho-mimic group resulted in decreased remodeling, microtubule density, and arrhythmia in a mouse DMD model when compared to mouse without a phospho-mimic group (2). This finding suggests there may be a relationship between the phosphorylation of connexin- 43 and the structure and function of cardiomyocyte. A subsequent study interested in investigating the increase in microtubule density present in DMD mouse concluded administration of colchicine, a microtubule polymerization inhibitor, resulted in a decrease in arrhythmias and cardiomyocyte remodeling. Currently, there is a gap in knowledge pertaining to the relationship between connexin-43 and microtubules. This study aims to use a phosphomimic mouse model to study the interaction between microtubules and connexin-43.

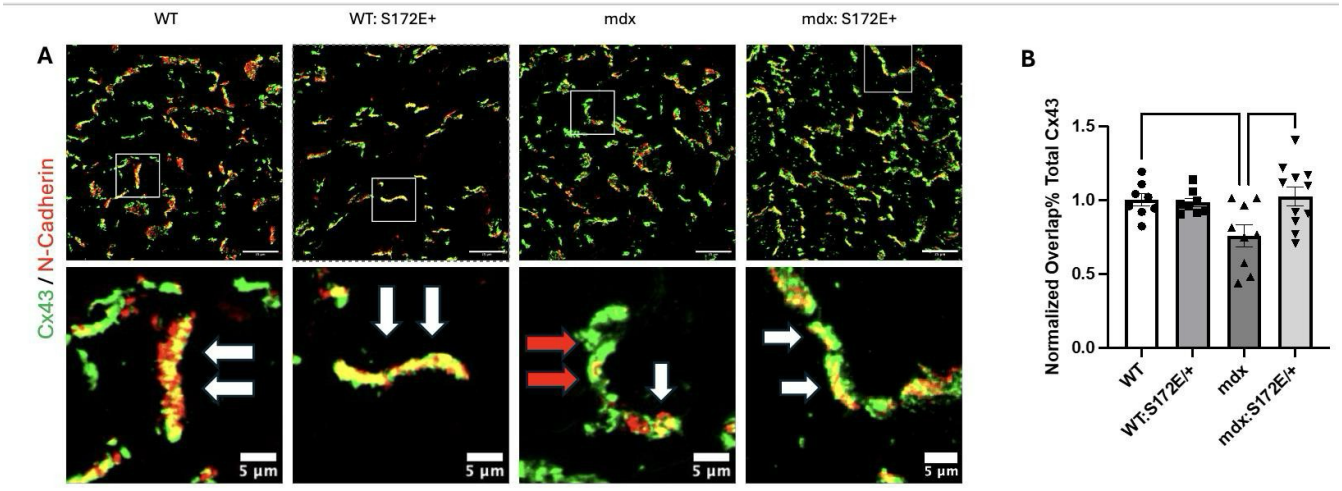
To examine the relationship between microtubules and connexin-43 requires the creation of a microtubule deficient mouse model to serve as a negative control. Through genetic editing, a mutant tubulin 3 with the serine at the 172 is replaced with a glutamic acid (tubulin 3 S172E) to mimic the effects of phosphorylation of the beta tubulin. The effect of phosphorylation inhibits binding of GTP binding to the tubulin alpha-beta dimer, this will arrest polymerization of the microtubule.

Method:

Immunofluorescent Staining

Examination of the microtubule-Cx43 relationship necessitated immunofluorescent staining of four mouse models: wildtype, wildtype S172E, DMD (mdx), and mdx S172E. All groups underwent a cardiac chemical challenge to induce accelerated progression of the dystrophic morphology. Isoproterenol, a beta-adrenergic agonist, was administered twice at a 7-day interval, the heart was harvested 1 hour after second dose. Sequentially, the cardiac samples were harvested and submerged in OCT blocks in preparation for cryosectioning. To denote the position of the intercalated disks, sections containing cardiac tissue were treated with N-cadherin targeted antibodies. A second antibody was used to target Cx43 molecules. Upon exposure to immunofluorescent light, these antibodies will emit red and green signals, respectively. Two-way ANOVA was used to compare the normalized overlap between Cx43.

Results:



Cx43 remodeling in the hearts of mdx: S172E/+ mice. **(A)** Representative confocal immunofluorescent images with enlarged insets of cardiac cryosections from 3-6-month-old mice stained with Cx43 (green) and N-cadherin (red). Scale bar = 25µm. The white arrow indicates the Cx43 at the intercalated disc, whereas the red arrow indicates the remodeled Cx43. **(B)** Quantification of Cx43 locate d d at the intercalated disc in confocal immunofluorescent images, the ratio of co-localized Cx43 over the total level of Cx43. processed in Fiji ImageJ. All data points are normalized to the average value of the WT group. n = 8 (WT, WT: S172E/+), n = 9 (mdx), n = 11 (mdx: S172E/+). Each dot represents a mean value of 3 images per mouse heart. Both sexes were used in a blinded fashion. Two-way ANOVA, Tukey's multiple comparison test. *P<0.05; **P<0.01. Data are presented as means ± SEM.

Analysis of the results were notable for significant changes in between the wildtype and mdx mouse model. Additionally, there was an improvement in the mdx: S172E when compared to the mdx model.

It is important to note that comparable Cx43 density at the intercalated in wildtype and

mdx models does correspond to rescue in cardiac function. In mdx mouse models, an overall decrease in Cx43 density localized at the intercalated disk causes an upregulation of Cx43 production. Unorganized protein trafficking in mdx mouse models and increased production facilitates Cx43 channels to be inserted at the intercalated disc and the lateral walls of the cardiomyocyte. Under immunofluorescent imaging, this will correlate with an increase in green signal in mdx mouse models accompanied by what appears to be normal Cx43 density at the intercalated disc.

Conclusion: The phosphomimic mutation, Tubulin 3 S172E, helps to reduce remolding of Cx43 in mdx heart at age of 3-5 months

Systematic Approach to Identify and Isolate Glioblastoma-Cancer Stem Cells

Student: Jesus Benites

Mentor: Pranela Rameshwar, PhD

Glioblastoma Multiforme (GBM) neoplasms are classified as grade IV astrocytomas and are the most aggressive form of glial cell cancers in the CNS. GBM generally arises from de-novo mutations or the potential advancement of grade I astrocytomas [1]. GBM remains local and does not metastasize to other organ systems mainly due to the blood-brain barrier. Although it lacks metastatic capabilities, GBM has a 5-year survival rate lower than 5% for middle-aged adults [2]. Despite the advancements in many other areas of oncology, GBM remains an underserved area of cancer research with few new therapies providing substantial improvements to patient survival rates beyond 5 years [3]. As such, investigation into new therapeutic regimens or novel drugs for GBM is of great interest to many in the field of translation cancer research. One way to address the high GBM mortality rate is by closely examining long-term cancer stem cells (LT-CSCs) and their role in relapse and cancer progression. GBM has the potential to relapse due to its self-renewal capacity as well as its ability to resist chemotherapy. Most of these two abilities are suspected to arise from this LT-CSC subpopulation within the tumor.

Our study aimed to use a SORE6-GFP reporter to select for GBM-LT-CSCs. The SORE6-GFP reporter contains tandem repeats of SOX2 and OCT4 promotor regions, both of which are well-established stem cell markers. We hypothesized that similar to previous findings in breast cancer cell lines, we would be able to create a CSC hierarchy using the SORE6-GFP reporter in GBM [4]. Using FACS we successfully isolated SORE6-GFP high, medium, and low populations. We were able to confirm that SORE6-GFP high are GBM-LT-CSCs by performing a tumorsphere assay using two GBM cell lines, U118 and T98G. We aimed to identify the effect of stress induction following recurring rounds of passaging on our GBM-LT-CSC populations.

We aimed to study the effects of culturing a heterogenous population of GBM cells under stressful conditions and observing the changes in stem cell gene expression following consecutive passaging. To create a stressful environment, we began by culturing adherent GBM cells from our two cell lines, U118 and T98G, in low-attachment 6-well plates. Once sphere-like structures appear, denoted as a minimum of 10 aggregated cells, a portion of cells are collected, while the rest are passaged. Cells were passaged about three times

and the cells collected at each passage were used to isolate RNA. It was then subjected to downstream analysis via RT-PCR to identify significant changes in stem-cell-related gene expression. This allowed us to establish the effects that our stressful environment had on the replication of GBM-CSCs.

In the results gathered, we noticed that both lineages did have self-renewing capabilities as they successfully grew after several passages. In addition, we noted an increase in stem cell markers SOX2, OCT4, and Nanog. Therefore, these promotor regions might play an important role in the viability of this tumor, making it a site of interest for future research.

Upon completing our study, we've hoped to pave the path to find other better ways to isolate and observe GBM-LT-CSCs. In addition, we can use what was studied and aid our understanding of the growing nature of GBM within the brain by using a GFP-sensitive scan on mice models [5]. Taking this a step further, we might be able to monitor how GBM cells respond to specific therapies as the scans would show us either its progression or regression. There is still more to learn about GBM; however, this can be the scientific push the field needs to fight against this deadly tumor.

Metabolomics in CardiovascularDisease

Student: Tariq Bullen

Mentor: Junichi Sadoshima, PhD

Cardiovascular disease (CVD) remains the leading cause of death worldwide, thus making prevention a realistic objective. Because of this, research has shifted into the investigation of cardiac myocyte longevity through the reduction of apoptosis, necrosis, and autophagy as they have been investigated as contributors to diminishing the heart's pump function and signaling thus being a major source of inflammation, fibrosis and other heart disorders. Thus, genetic mouse models are frequently used to investigate the role of cardiac myocytes in biochemical, cardiac specific signaling assays, proteins and genes (through transduction with adenoviruses) in cardiac function, protection, and distress such as ischemia and heart failure.

The long term objective of this work is to be able to selectively manipulate cardiac proteins and genes to stop or lower apoptosis, necrosis and autophagy, in order to conclusively produce therapeutic treatments for inflammation, fibrosis and other heart disorders. The short term objective is to better understand and identify cardiac specific proteins and genes involved in the role of myocyte signaling.

Hypothesis: Under stress, the presence of specific proteins and genes predominantly mediate cell death via a series of cascade signaling of, if not one, but three types of phenomena: apoptosis, necrosis and autophagy, which has been link to myocardial cell destruction and, as a result, cardiovascular disease.

Specific Aim: During prolonged chronic inflammation or cardiac fibrosis we are going to determine if autophagy is a suicide pathway or a sign of failed cardiomyocyte repair that is directed by specific inflammatory cytokines and chemokines. This will be accomplished by generating myocardial infarction in neonatal rat hearts in order for fibrosis to develop. The isolation of myocytes from neonatal rat hearts are centrifuged, precipitated and grown on a medium to investigate certain proteins like integrins, a set of signaling molecules often studied in fibroblast activation, that may have direct or indirect connection to myocyte longevity.The transduced signaling cascades that promote a migratory, matrix-synthetic and proliferative fibroblast phenotype [1] as well as the protein coupled receptors and growth factor receptors that activate downstream pathways that promote matrix

transcription [2] will be blocked or turned off so that fibrogenic macrophages and lymphocytes may cause cardiomyocyte necrosis, triggering a reparative form of fibrosis via autophagy. The damaged cardiomyocytes that show characteristics of autophagy will be examined and mediated under specific conditions to discover which genes may be linked

Impacted: Ultimately, this study opens possibility for creation of new therapeutic approaches for heart conditions that can reverse degeneration of cardiac tissue curing patients of cardiovascular diseases and extending life expectancy

Aspirin Mediated Restoration of MSC's Via TGFβ

Student: Olafotun Ebunlomo

Mentor: Pranela Rameshwar, PhD

Transforming Growth Factor-Beta (TGF-beta) signaling pathway has been implicated in the mechanism through which aspirin induces Mesenchymal Stem Cells (MSCs) to exhibit normal behavior. Upon aspirin stimulation, there is a notable upregulation of TGF-beta expression accompanied by an increased release of this signaling molecule. While experimental data has shown alterations in TGF-beta levels upon aspirin stimulation, the causative relationship remains to be elucidated. To address this gap, we initiated a study aiming to upregulate TGF-beta expression in preeclampsia cells, a context where MSCs display aberrant behavior. Our approach involved the growth of TGF-beta plasmids followed by the transfection process. Currently, we are in the selection phase, utilizing antibiotics to isolate cells carrying the TGF-beta plasmid. While no data is available at this stage, this transformation from plasmid growth to transfection marks a crucial step in our investigation. This research not only sheds light on the intricate interplay between aspirin, TGF-beta signaling, and MSC behavior but also holds potential implications for therapeutic interventions targeting abnormal MSC function in conditions such as preeclampsia.

Testing the dependance of known alarmins on NFAT signaling and identifying additional NFAT dependent inflammatory mediators that play a role in the disease process of asthma.

Student: Carlos Garcia Colon

Mentor: Darren Wiesner, PhD

Allergic asthma continues to place a significant burden on a growing number of individuals, especially children, in the United States as well as in the healthcare systems that care for them. This condition is mediated by a disproportionate response to particular allergens that trigger Th2 mediated inflammatory responses in the lungs leading to various well-understood effects such as bronchoconstriction, increased mucus production, and long-term airway remodeling if not properly controlled. While these downstream effects are well-described, the mechanisms underlying the sensitization process and resulting signaling cascades that trigger the development of the inflammatory environment are far less well understood. Notably, the particular cytokines, alarmins, and chemokines involved in the initial generation of this inflammatory environment and their regulation have yet to be fully characterized, which limits our understanding of this response and our ability to reliably target or affect it.

Previous research has shown that asthmatic response to common fungal protease allergens is mediated by epithelial tight junction damage. This damage is sensed by TRPV4, a mechanosensitive member of the transient receptor protein family found predominantly in club cells that triggers pro-inflammatory calcium dependent signaling via calcineurin in the lung epithelium. We hypothesized that the inflammatory response to protease allergens in lung epithelium involves transcriptional regulation by NFAT and additional distinct NFAT dependent inflammatory mediators. In order to test this hypothesis, we challenged lung epithelial cells with Alkaline phosphatase 1 (ALP-1), a fungal protease secreted by all members of the Aspergillus genus, that serves as a well known allergen that can trigger asthma in sensitized individuals. After this ALP-1 challenge, we established a protocol to measure the release of proinflammatory cytokines (IL-5, IL-33, and CCL2) in the culture medium. After identifying the levels of proinflammatory cytokines present, we continued to determine if these effects were NFAT mediated by repeating the challenge in cultures treated with cyclosporine, a known calcineurin inhibitor that disrupts NFAT signaling. This step of the project is ongoing.

We focus on how the pulmonary epithelium reacts to common allergens because it has been shown to be an integral aspect of asthmatic sensitization. The epithelium serves as an significant exposure surface to all inhaled substances, and understanding how it reacts to certain irritants can provide crucial information about the early stages of this disease process. Going forward, the long term goal of this project is to acquire a more comprehensive understanding of the events that trigger asthmatic sensitization to particular allergens which would allow us to identify new potential targets for intervention and hopefully reduce disease burden in susceptible populations.

The Relationship between Dental Pulp Mesenchymal Stem Cells and Leukemia

Student: Mark Ibrahim

Mentor: Ioanna Tsolatki, PhD

Introduction: Within bone marrow, hematopoietic stem cells (HSCs) and bone-marrow Mesenchymal Stem Cells (bmMSCs) are both responsible for the generation of new cell lineages from progenitor cells. Cancer cells that have infiltrated the bone marrow can utilize stem cells and their unique environment to further promote their own growth.

Cancer cells interact with the unique environment of stem cell tissues through intracellular communication. One such method of information transfer is through the use of gap junctions, a form of cell contact. In gap junctions, connexin proteins form large transmembrane proteins responsible for Gap Junction Intracellular communication (GJIC). Centering chemotherapeutic drug development around connexin may not be favored as connexins are often utilized in other biological processes.

There exists an additional subtype of MSCs comprised of multipotent cells that reside in the dental pulp of teeth. These Dental Pulp Mesenchymal Stem Cells (dpMSCs) share properties with bmMSCs, and their interaction with cancer cells is a novel area of research. Leukemia is known to infiltrate the oral cavity relatively early in its pathogenesis, and the interaction between Leukemic Cancer Stem Cells and dpMSCs has not yet been characterized. Utilizing the mesenchymal stem cells found in dental pulp (dpMSCs), we can investigate how the cancer cells interact with the oral environment and potentially pinpoint areas for future research and drug development.

Our long term goal is to characterize the interaction between myelogenous leukemia cells and dpMSCs, and elucidating any mechanisms or pathways in this relationship that may serve as potential therapeutic targets.

Objective: We will explore the relationship between dpMSCs and Leukemia by performing A dye transfer assay to assess intracellular communication between the leukemic cells and the dpMSC’s.

Methods: As established in previous research, gap junctions provide an area of cytoplasmic exchange between the two complexed cells, allowing for some cytosolic components to move from one cell to the other using the Connexin-formed pores. If either the dpMSCs or the Leukemic cells could be labeled with a well-retained cytoplasmic dye,

cytoplasmic exchange between these two cells could be observed and disruption of GJIC pathways would likewise prevent any such dye exchange. 1-Octanol is a specific inhibitor of Connexin43, which is vital in the formation of GJIC structures.

This exchange of dye can be analyzed using both a fluorescence microscope and a flow cytometer. Briefly, the dpMSC's are labeled with CellTacker CMAC dye (microscopy) or Deep red (Flow Cytometry) by incubation in 10uM dye media for 30 minutes. They are then directly co-cultured with Leukemia cell lines (HL60 or K562) at a 1:1 ratio in complete culture media, with half of the wells being given 1-Octanol to inhibit

Connexin-mediated GJIC formation. After co-culturing, imaging studies are done to assess the dye transfer. If the dye has transferred, then the leukemic cells have established GJIC's with the dpMSC's. Likewise, if dye is observed in the Leukemic cells in the complete media condition but not in the conditions containing 1-Octanol, then this would be further confirmation that cytoplasmic exchange occurs via gap-junctions.

Results: If Leukemia cells and dpMSCs are forming gap junctions between each other, then dye should move from the Leukemia cells into the dpMSCs and 1-Octanol as an inhibitor of Connexin43 should be able to inhibit the formation of these junctions. However, we observed that the dye exhibited transfer between the dpMSCs and the Leukemic cells; however, the presence of 1-Octanol failed to inhibit this transfer.

Conclusion: The observed transfer of dye between the dpMSCs and the Leukemic cells suggests communication via gap junctions. However, the ineffectiveness of 1-Octanol in inhibiting this transfer indicates that Connexin43 is not the specific protein facilitating these gap junctions and further testing is required.

Characterization of RAD50 Mutations on RAD53 Phosphorylation

Student: Charles Ma

Mentor: Katsunori Sugimoto, PhD

Objective: DNA double-stranded breaks (DSBs) are a form of DNA damage that can arise from exogenous or cell-endogenous processes. DSBs caused by exogenous processes, such as exposure to irradiation or chemicals, are potentially lethal to eukaryotic cells if essential genetic information is lost at the break site, while those produced by cell-endogenous processes generate essential intermediates are required for programmed gene rearrangements in the vertebrate immune system and homologous recombination. These DSBs can be repaired through one of two mechanisms: non-homologous end joining (NHEJ) and homologous recombination (HR). Upon formation of DSBs, protein complexes that specifically recognize these lesions localize to these sites. The Ku heterodimer (Ku70-Ku80) binds to the ends of the DSBs. In HR, a protein complex consisting of Mre11, Rad50, Nbs1/Xrs2 (MRN/X) is recruited to the DSB site. Mre11 is the nuclease, Rad50 is the ATPase, and Nbs1/Xrs2 is the DSB localization domain. The MRX complex recruits Tel1/ATM kinase via the Xrs2 C-terminal region. Tel1/ATM activates Rad53 protein kinase, which stimulates DNA damage checkpoint signaling, and phosphorylates Sae2, which stimulates Mre11 exonuclease activity.

The MRX complex can change between the ‘closed’ (inactive) to ‘open’ (cutting) conformation when ATP hydrolysis by Rad50 occurs. When Rad50 is bound to ATP, Mre11 3’→ 5’ exonuclease activity is inhibited. Phosphorylated Sae2 stimulates Mre11 exonuclease activity in the MRX complex. In Sae2 deletion mutants, after hairpin-capped DNA ends are induced using an HO endonuclease system with an inverted repeat sequence, Mre11 is unable to repair the DSB and Tel1 signaling is upregulated, which upregulates Rad53 phosphorylation. Recent work indicates that Rad50 mutants that fail to interact with phosphorylated Sae2 also results in decreased nuclease activity, which is correlated with increased Rad53 phosphorylation. Our objective was to determine the effects of different Rad50 mutations on Tel1 activation, as monitored by phosphorylation of Rad53.

Methods: Yeast containing the Rad50-47 mutation and Rad50-126 mutation were cultured by Dr. Sugimoto. The HO endonuclease system was used to generate DNA double-stranded breaks (DSBs). After HO induction of DSBs, cells were lysed and Rad53 phosphorylation was assayed via Western blot using an anti-Rad53 primary antibody followed by an Odyssey-IR secondary antibody for imaging.

Summary (Figure 1): In a single experiment, the absence of HO-induced DSBs showed no shift in the band of Rad53, suggesting lack of phosphorylation. Using extracts from HO-induced cells with double Sae2 Mec1 deletion mutants and Rad50-126 and Rad50S mutants, there were band shifts, as compared to the corresponding negative controls without HO induction.

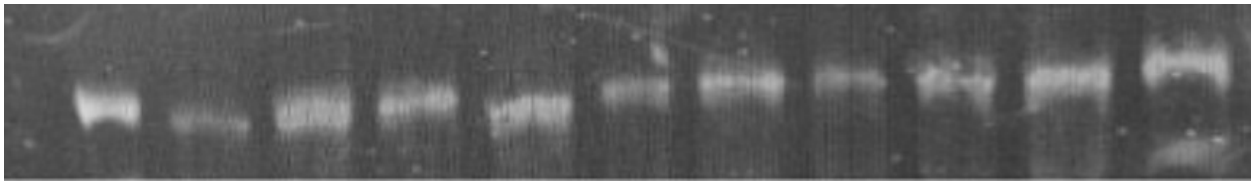


Figure 1. Western blot of wild type (WT) and yeast mutations containing Sae2 and/or Mec1 deletions or mutations in Rad50.

Lane	Sample	HO Induction	Band Shift
1	Molecular Ladder (100kDa)	N/A	N/A
2	WT (SAE2 MEC1)	-	-
3	WT (SAE2 MEC1)	+	-
4	*Δ*sae2 *Δ*mec1	+	+
5	WT(SAE2) *Δ*mec1	+	-
6	Rad50-47 mutant	-	-

7	Rad50-47 mutant	+	-
8	Rad50-126 mutant	-	-
9	Rad50-126 mutant	+	+
10	Rad50S mutant	-	-
11	Rad50S mutant	+	+

Conclusion: Due to a single experiment, one cannot conclude that different Rad50 mutations on Tel1 activation, as monitored by phosphorylation of Rad53. Future research studies will require additional experiments.

Participation Description: Charles Ma ran the SDS-PAGE gel, transferred proteins to a membrane and incubated the membrane with anti-Rad53 primary antibody only once.

Other parts of the experiment, including cell cultures and image capturing. were done by Dr. Sugimoto. A web-based notebook was set up, but none of the experiments were recorded in the notebook. The image (Figure 1) was obtained from Dr. Sugimoto.

Do dental plaque microbial profiles differ across various antiretroviral therapy regimens?

Student: Alekhya Thota

Mentor: Modupe Coker, PhD

Objectives: Patients living with HIV, despite undergoing antiretroviral therapy (ART) regimens, are prone to systemic oral pathologies including dental caries. The cause of this increased risk is not fully understood. Nevertheless, HIV, even when treated, causes a low-grade inflammation and dysbiosis environment in the oral cavity. This study aims to examine plaque microbiota and their differences in patients treated with various ART regimens.

Methods: This was a cross sectional study of children living with HIV, who differed by the ART regimen they received. The regimens were analyzed based on two different groupings. Two categories of ART regimens were based on drug types. First group was based on the nucleotide reverse transcriptase inhibitor (NRTI) of the ART regimen, namely: Azidothymidine (AZT), Abacavir (ABC), or Tenofovir DF (TDF). The other group was based whether the regimen had non-nucleotide reverse transcriptase inhibitors (NNRTI) i.e., nevirapine and efavirenz, or not. Regimens without NNRTIs included integrase/protease inhibitors. Supragingival plaque samples were collected and classified according to the overall oral health of the child (caries-active or free) and the health of the individual tooth (ICDAS annotation). Each plaque sample was sequenced using a novel meta taxonomic approach targeting a fragment of the bacterial rpoC gene.

Results: Caries status of the patient, type of tooth that the plaque was collected from, as well as viral load were factors that were associated with the plaque microbiome. However, there was no difference in terms of the regimens; NRTI groups and the NNRTI groups were not significant in terms of diversity. However, the ART duration was significant (Figure 1). Therefore, Plaque microbiota differ more so with ART duration rather than class of ART regimen, when controlling for factors such as age and viral load. Participants who had been on ART longer tended to have a more diverse microbiome. Furthermore, ART regimen was significant only in the viremic population, and was not apparent in the total sample population. Bacteria such as *Rothia mucilaginosa* and *Capnocytophaga sp. Oral taxon 412* were similar between the total sample population and the viremic group, indicating that they may be driven by HIV or ART (Figure 2).

Conclusions: Plaque microbiota differ more so with ART duration rather than ART regimen, when controlling for factors such as age. Additionally, patients who had a longer ART duration tended to have a more diverse microbiome. ART regimen was significant only in the sample of patients who were viremic and was not apparent in the total sample population. Further investigating the microbiome's link to pathophysiologic changes is essential in forming HIV therapies for patients, keeping in consideration the low-grade inflammation and dysbiosis of the oral microbiome that may occur.

Table 1: Summary of Sample Population statistics (n=146)

Males, n (%)	65(45)
Average CD4 count	743.7 counts per mm^3
Average age	7.2 years old
Regimen NRTI-based group (group 1), n(%)	
AZT	119(82)
ABC	18(12)
TDF	9(6)
Regimen NNRTI-based group (group 2), n (%)	
EFV/NVP	123(84)
DTG/LPVr	23(16)
CD4 lymphocyte in mm3, mean (SD)	969(533)
Detectable HIV-1 Viral Load, n (%)	53 (36)