**RNA sequencing and proteomics analysis to identify molecular pathways implicated in the development of aortic aneurysms**

**Project Name:** RNA sequencing and proteomics analysis to identify molecular pathways implicated in the development of aortic aneurysms

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**Project GitHub Repository:** [bmsip\_internship\_seta](https://github.com/KyraGriffin/bmsip_internship_seta)

**Background**

Cardiovascular diseases remain the leading cause of morbidity and mortality in the USA (Fry et al., 2016).  Aortic aneurysms are a vascular condition that currently has no cure or treatment. The aneurysms are abnormal enlargements of the aorta that can occur in the thoracic and abdominal region (Seta, 2022). These abnormal dilations are usually associated with genetic mutations and hypertension and affect about 80,000 Americans a year.

Dr. Seta’s research focuses on cellular and molecular mechanisms of vascular diseases to identify novel therapeutic targets. The laboratory focuses on the transcription factor Bcl11b and the deacetylase Sirtuin-1 and uses genetic mouse models to examine their role in the pathology of aortic aneurysms. Sirtuin-1 is a nicotinamide adenine dinucleotide (NAD+)-dependent class III histone deacetylase expressed in a wide range of tissues, including the vasculature. It regulates many cellular processes, including cell survival, apoptosis, inflammation, stress resistance, cell growth, cell senescence and metabolism. Previous research has identified that Sirtuin-1 is involved in maintaining the integrity of the aortic wall, which eventually breaks down when Sirtuin-1 is deleted (Fry et al., 2015; Budbazar et al., 2021).

**Study Design**

The Seta lab developed Sirtuin-1 smooth muscle specific knockout mice (SMKO) and induced stress using a hypertensive agent, angiotensin II (angII). Examination of the aortas showed that the angII-treated SMKO had disorganized elastin lamellae with frequent elastic breaks compared to the angII-treated-WT. The presence of these very disorganized lamellae explains why the SMKO mice are more likely to suffer aortic tearing.  Their work also found increased extracellular matrix-degrading enzyme activity of the matrix metalloproteinases 2 and 9 (MMP2, MMP9) in the aortas of the angII-treated SMKO mice but found that the angII-induced increases in MMP2 and MMP9 activities were prevented entirely in mice overexpressing SirT1 in the vascular smooth muscle, VSM.

Sirtuin-1 has been shown to target a variety of transcription factors, such as Nrf2, p66Shc, NF-B, p21, and p53, which are involved in various cellular processes such as energy balance, DNA repair, replicative senescence, and apoptosis. In order to identify VSM-specific mechanisms. they performed a mass spectrometry proteomics analysis on the acetylated lysine-enriched aortic samples of five WT, five SMKO, and five SMTG (transgenic overexpression of SirT1) mice treated with angII. The flow-through of the acetyl lysine-enriched aortic samples was also analyzed via mass spectrometry to determine the overall protein expression changes downstream of SirT1 in the aorta after angII.

To identify molecular mechanisms and role of SirT1 in aortic aneurysms, I will perform bioinformatic techniques to find differentially abundant proteins between the wild-type, knockout, and transgenic mouse models with the goal of identifying direct targets of SirT1. In addition, the total proteomics data will be used to explore secondary changes in protein expression and networks regulated downstream of SirT1. Identification of these direct or indirect changes may lead to potential therapeutic targets or pathways for the treatment of aortic aneurysms.

The proteomics data will be analyzed using various techniques. For example, differential abundance, visualize protein networks, specifically STRING - Protein-Protein Interaction (PPI) and Ingenuity Pathways Analysis (IPA), gene ontology analysis, and differential expression of proteins between knockout, wildtype, and transgenic mice models. As of now we plan to do a preliminary analysis utilizing a T-test with multiple hypothesis correction (FDR), then we will conduct differential expression analysis using limma (Ritchie et al., 2015).

The RNA seq data consists of WT, WT/angII, BSMKO (mice with Bcl11b deletion), and BSMKO/angII with five mice per group. If we have time once the proteomics analysis is relatively complete, the RNA seq analysis will be performed. If we do conduct analysis of the RNA seq data, I will first be performing fastqc, then do adapter trimming, multiqc, and alignment of the RNA seq to the mouse genome mm10 using star or bowtie2. Once we have aligned our samples to the genome, we can perform differential gene expression analysis using deseq2 and we can utilize a small batch correction when performing this analysis (Love MI et al., 2014).

**Scope and Responsibilities**

The Seta Lab will provide proteomics results for the acetylated lysine-enriched aortic samples and comprehensive proteomics analysis of the flow-through (i.e., the total proteome without the acetylated protein fraction). The Seta lab will also share RNA seq data that was obtained at the Genomics Core at Harvard University. As the intern on this project, I will be responsible for performing the bioinformatics techniques and analysis and communicating with the PI and mentor to analyze the data and interpret the results in a biological context.

**Project Timeline**

A description of the anticipated timeline of the project, including the estimated calendar time to completion for each item. Organized roughly by week.

Complete the proposal and get familiar with the background of the project (weeks 1 & 2)

Environment setup and preliminary analysis (week 3)

Comprehensive preliminary differential expression analysis (week 4)

Perform preliminary protein network analysis (week 5)

**References**

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4. Liu, Y., Song, Y., Liu, P., Li, S., Shi, Y., Yu, G., ... & Zhu, W. (2021). Comparative bioinformatics analysis between proteomes of rabbit aneurysm model and human intracranial aneurysm with label-free quantitative proteomics. CNS neuroscience & therapeutics, 27(1), 101-112.
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