Cassie Heilingoetter Specific Aims

Fibrodysplasia ossificans progressiva (FOP) is a disease that causes excessive and irreversible bone formation at soft tissue sites such as muscles, ligaments, and connective tissue. [1,2,3,4] Moreover, soft tissue damage from surgery, intramuscular immunizations, or even a bump can induce bone growth at joints that permanently restricts local movement.[4,5,6] FOP is caused by a single missense mutation in the 206th amino acid (R206H) within a transmembrane protein called activin A receptor type I (ACVR1). [3,4] The mutation resides in the TGF-β domain of the ACVR1 protein. ACVR1 is a receptor in the bone morphogenetic protein (BMP) family.[2] Under normal conditions in development, the BMP pathway causes changes in gene expression that induce the formation of bone and cartilage.[7] ACVR1 directly activates BMP signaling when bound to its ligand, ultimately causing the downstream changes that initiate bone development. When ACVR1 is mutated, based on the disease phenotype, the BMP signaling pathway appears to be "leaky" and facilitates BMP signaling in cells that are not supposed to be forming bone.[1,3,7] Ultimately, this aberrant signaling turns endothelial cells into mesenchymal stem cells and subsequently into bone. Although it is known that ACVR1/BMP signaling is disrupted in FOP, the molecular mechanisms that facilitate the over-stimulation of this pathway are not fully understood.

The **hypothesis** is that the missense mutation in the TGF-β domain confers ACVR1 signaling to be constitutively active by disallowing an inhibitor protein, called FBPK12, to bind to ACVR1. This causes increased receptor signaling activity, which stimulates changes in gene expression and cell differentiation. *In vitro* and protein modeling studies have suggested that the mutation changes the inhibitor binding affinity; however, no *in vivo* studies testing this have been conducted. The **primary goal** of this research is to better understand the genes involved in the conversion of soft tissue into bone, as well as the role of the inhibitor. The **long-term goal** is to understand the molecular mechanisms involved in FOP to ultimately develop treatments.

Aim 1: Perform an expression comparison of muscular tissue in the normal and pathological state. **Approach:** Using a microarray, compare transcripts and gene expression between normal mouse muscle tissue and damaged ACVR1 mutant mouse tissue. **Hypothesis:** I would expect gene expression changes related to BMP signaling to be active in the pathological state, confirming that the disease is a result of constitutive activation of ACVR1 signaling. **Rationale**: This will be used to determine which genes are active in the pathological vs. normal state. Additionally this will indicate what genes are involved in transdifferentiation from muscle to bone.

Aim 2: Determine if using chemical genetics to inhibit the aberrant receptor enables the rescue of normal gene expression. **Approach:** Screen the inhibitors found on the Pubchem database for ACVR1. **Hypothesis:** I expect if the disease is caused by leaky activation of ACVR1, that using inhibitors could potentially rescue the aberrant signaling. **Rationale:** Successful inhibitors can be a potential drug therapy. Perhaps inhibitors can help diminish the uncontrolled activation and restore gene expression to normal conditions.

Aim 3: Compare ACVR1 post-translational modifications at the 206th amino acid in the disease state vs. the wild type state. **Approach:** Determine peptide size with hypothetical trypsin cuts using Expasy then run SDS page gel and follow up with tandem mass spectrometry for additional phosphorylation sites. **Hypothesis**: I expect if phosphorylation is responsible for the decreased binding affinity of the FKBP12 inhibitor that there will be a shift in the weight on mass spectrometry for the fragment containing the mutation. **Rationale:** This may determine whether or not the mutation causes the FKBP12 inhibitor to have decreased binding affinity for the TGF- β domain because of a new phosphate group or not.

This project is crucial due to the fact that it is not clear how these cells are undergoing their metamorphosis. It is expected that the outcome of this study will help to identify genes that are misregulated in FOP and isolate drug targets to limit aberrant signaling. In the long term, the hope is that this study will inform the development of effective treatments for FOP.

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