**Alcohol Intolerance** is a disease that disrupts the alcohol degradation pathway (1). There are multiple enzymes that could affect metabolism, but the most common interruption is in the acetaldehyde dehydrogenase-2 (ALDH2) enzyme. ALDH2 is highly localized to the liver where acetaldehyde is converted to harmless acetate (2). This protein has one domain, aldedh, which reduces aldehydes. Most people with this disease avoid alcohol due to the adverse reaction known as alcohol flush. The most common polymorphism at the 487Glu site of ALDH2, known as the E487K mutant (2,3). Approximately 8% of the world’s population has at least 1 copy of the mutant ALDH2 allele, and will result in an inflammatory response to the acetaldehyde accumulation in the blood (2,4). Overall cancer risk in ALDH2 mutants is significantly higher than the homozygous wild-type counterpart (6). There is a significantly higher incidence of oral, head, neck, and liver cancers (7). *It is still unknown how ALDH2 causes cellular proliferation, especially in liver cells.*

My **primary goal** is to determine the contribution of ALDH2 to cellular proliferation, specifically at its primary active site in mouse hepatocytes. My **hypothesis** is that ALDH2 interacts with cancer causing genes or proteins that causes an abnormal cell lifecycle. I plan to use a mouse model due to the homology of the digestive system, and the presence of liver tissue. The alcohol metabolism pathway could be affected by multiple different factors, such as diet, so it is very important that my model is very homologous to humans overall. My **long-term goal** is to understand how ALDH2 contributes to cell cycles abnormalities in other tissues, including the tissues that do not have significant interactions with the alcohol degradation pathway.

**Aim 1**: Determine conserved amino acids that are essential for ALDH2 function.

*Approach*: Using Clustal Omega, conserved amino acids from the ALDH2 homologs will be evaluated based on their ability to process alcohol. These amino acids will be mutated in mice using CRISPR/Cas9 to have an inactive form ALDH2. Mice will be assayed based on their ability to process ethanol by testing acetaldehyde levels in blood after alcohol consumption.

*Hypothesis*: Changes in the aldedh domain will result in inactive or less active form of ALDH2.

*Rationale*: Not all mutations in ALDH2 has the same impact on alcohol metabolism. Therefore, analyzing the mutations in conserved sequences will determine the mutations in ALDH2 that are essential for metabolism.

**Aim 2:** Identify differentially expressed genes in mutant ALDH2 mouse hepatocytes.

*Approach:* Create a mutant mouse line with the E487K mutation and detect it by showing an immune reaction to alcohol consumption. Then, perform RNA-seq on wild-type and mutant ALDH2 to determine differentially expressed genes.

*Rationale:* Determining differentially expressed genes will help identify target genes that are essential for a normal cell cycle. Abnormal expression of oncogenes and tumor suppressors is the driving cause of cell proliferation.

*Hypothesis:* Known oncogenes or tumor suppressors will have differential expression, which will cause cell proliferation in the mutant mouse line.

**Aim 3:** Characterize protein interactions that are important for normal liver development.

*Approach:* Mutants created in Aim 2 will include a BioID tag in the transgenic organism. Then, hepatocytes will be extracted from the mouse models and grown under BioID conditions. Biotinylated proteins will be extracted and sequenced.

*Rationale:* Characterizing the interactome of ALDH2 will determine how the mutant ALDH2

*Hypothesis:* ALDH2 will biotinylate nearby proteins that may be affected by ALDH2 or its substrates.

**Future Directions:** Assay other tissues that are commonly affected by cancer in ALDH2 tissue. For example, head and neck tissue also have increased incidence of cancer risk in mutants. Much is also unknown about ALDH2 and colorectal cancer.

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