Tyrosinemia Type 1 is a rare autosomal recessive disease that directly impacts liver and kidney health, often leading to liver cirrhosis and hepatocellular carcinoma, while also displaying a vast number of other symptoms. [1] The three types of tyrosinemia are each classified by a different mutated gene. Type 1 is attributed to a mutation within the FAH gene that encodes for the enzyme fumarylacetoacetate hydrolase, which plays a large role in the catabolic tyrosine pathway and breakdown of tyrosine byproduct fumarylacetoacetate throughout the body, but specifically in the liver.[2] With this enzyme absent, patients have a buildup of this protein and its breakdown product succinylacetoacetate (SUAC). This causes many complications, and while the effects on the kidney and liver have been well-studied and understood, *it is unknown exactly how fumarylacetoacetate and succinylacetoacetate buildup causes cell cycle arrest and apoptosis, leading to liver cirrhosis.*

My **primary goal** is to determine how *succinylacetoacetate buildup* facilitates the *cell cycle arrest and apoptosis* found in patients exhibiting liver cirrhosis. *Danio rerio* will be used as a model organism in this experiment due to its prominent similarities to the human liver in terms of function and structure as well as the added benefit of transparency and rapid development. [3] With this study, I **hypothesize** that excess succinylacetoacetate disrupts a pathway present in a normal functioning liver and causes hepatocyte cells to be unable to move forward through the cell cycle, causing increased apoptosis and in turn, cirrhosis of the liver. The **long-term goal** of this research is to further understand the mechanisms underlying this disorder in order to be able to effectively target symptoms with treatment drugs.

Aim 1 : Identify gene alterations that are linked to cell cycle arrest and apoptosis in the presence of succinylacetoacetate.
Rationale : Understanding how genetic changes due to excess SUAC presence contributes to cell cycle arrest and apoptosis is important as many metabolic processes contain SUAC as intermediates. In order to treat metabolic diseases that cause a rise in SUAC, looking at how cells respond on a molecular level is essential for determining SUAC implications on cellular function as well as to assess treatment options. **Approach :** Hepatocyte cells will be evaluated at different points in the cell cycle. CRISPR will be used to induce a mutation in the FAH gene of half of the population, and the control will remain untouched. Both of these groups will be monitored through the cell cycle, collecting samples at multiple times during the cycle to detect induced cell cycle arrest/apoptosis. DNA will be extracted and genome sequencing will be used to identify alterations in the genome at different points. This will link the genetic alterations to the point in the cycle at which arrest/apoptosis occur and further genome analysis can occur. **Hypothesis :** Organisms with a mutation in the FAH gene that went through cell division in the presence of excess succinylacetoacetate will have different genetic makeups/expressions at different points in the cell cycle than those observed without the mutation.

Aim 2 : Determine which checkpoint in the cell cycle hepatocyte apoptosis occurs when excess succinylacetoacetate is present.
Rationale : Determining the stage of the cell cycle at which induced hepatocyte apoptosis generally occurs in the presence of excess SUAC allows for mechanisms of this apoptosis to be further established. Knowing that apoptosis occurs at a particular point in the cell cycle would allow for checkpoint-specific regulators and pathways to be targeted to optimize treatment options. **Approach :** Hepatocyte cells will be treated with SUAC to promote apoptosis, while the control group is left untouched. RNA will be extracted at multiple points during the cell cycle and RNA-sequencing will be used to assess gene expression at different points during the cycle (to further confirm tests run in aim 1). Analyzing previously known cell cycle markers to determine changes in the expression will allow us to know at what point the apoptosis occurs. This will be further interpreted by using flow cytometry and fluorescently tagging the known cell cycle markers observed from the RNA-seq experiment where apoptosis occurs. **Hypothesis :** Hepatocyte apoptosis will occur late in the cell cycle, during the G2 checkpoint, as damage to the DNA will not allow it to pass through this checkpoint.

References

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