Kindler Syndrome (KS) is an autosomal recessive disorder that results in skin blistering and fragility, UV sensitivity, skin discoloration and blood vessel accumulation (poikiloderma), and potential development of squamous cell carcinoma.[1] KS is caused by a mutation in the FERMT1 gene, which encodes the kindlin-1 protein. Cancer development in KS patients occurs later in life, but the reasons for higher incidence of cancer is unknown.

The **objective** of this study is to identify the interactions that could explain why KS patients develop squamous cell carcinoma. Danio rerio will be the model organism used to study the condition because they show skin cancer-like and KS-like phenotypes. [2] The **hypothesis** for this study is that FERMT1 interacts with transcription regulation proteins and disruption of this interaction causes cancer development. The **long term goal** of this study is to uncover the reasons for cancer development in KS patients to potentially uncover preventative treatments for patients.

**AIM1: Identify highly conserved residues of FERMT1 in zebrafish via sequence alignment**

**Rationale:** In order to ensure the zebrafish model will accurately represent Kindler Syndrome, highly conserved residues of the kindlin-1 protein need to be identified. This will ensure that the mutants produced will have protein abnormalities like that of KS patients. **Approach:** The sequence of human kindlin-1 and zebrafish kindlin-1 will be aligned in MEGA using ClustalW for the sequence alignment. Since there is not a specific domain of kindlin-1 that is mutated in KS patients, a conserved region of each of the three domains of the protein will be mutated via CRISPR-Cas9. This will be done to try to identify if a mutation in a specific region of FERMT1 could contribute to cancer development. **Hypothesis:** Kindlin-1 is a highly conserved protein in most species. There will be an abundance of conserved residues in each of the domains of kindlin-1 to mutate. This will result in the establishment of three mutant lines of zebrafish that model KS (one for each domain of kindlin-1): kindlin-2-N mutants, PH mutants, and FERM mutants.

**AIM2: Analyze protein interaction networks of kindlin-1 in FERMT1 mutants via BioID Rationale:** Kindlin-1 has been shown to interact with a wide variety of proteins and be involved in many cellular processes. One of these proteins is CDC5L, which is involves with transcription and interacts with many transcription regulation proteins. **Approach:** The protein interaction network of the three mutant zebrafish models will be analyzed via BioID proteome analysis of kindlin-1. This will be done in adult fish because KS patients do not develop cancer until later in life. Mutants that develop cancer will be noted. **Hypothesis:** The interaction network of kindlin-1 will remain fairly like the WT in each of the three mutants. However, there should be a decrease in interaction with CDC5L in mutants overall and greater decrease in mutants that develop cancer.

**AIM3: Analyze the transcriptome of kindlin-1 in FERMT1 mutants via single-cell RNA sequencing Rationale:** Disruption of transcription regulation in FERMT1 mutants can be further proven by transcriptome analysis. Mutant phenotypes in overall expression would further prove kindlin-1’s role in transcriptional regulation. **Approach:** Single-cell RNA sequencing will be used to analyze transcription levels in dermal cells of adult zebrafish mutants. **Hypothesis:** There will be significantly different expression levels in the dermal cells of FERMT1 mutants compared to that of WT fish. Those fish that develop tumors will show exceptionally deviant levels.

**References**

1. Kindler Syndrome. (2020, January 21st). Retrieved February 4th, 2020, from https://ghr.nlm.nih.gov/condition/kindler-syndrome.

2.Postel, R. et. Al. (2013, September). *Kindlin-1 Mutant Zebrafish as an In Vivo Model System to Study Adhesion Mechanisms in the Epidermis.* Journal of Investigative Dermatology. Vol. 133, Issue 9. Retrieved March 2nd, 2020, from https://www.sciencedirect.com/science/article/pii/S0022202X15364071?via%3Dihub

3. What is Integrin? (2018). Retrieved March 2nd, 2020, from https://www.mechanobio.info/what-is-mechanosignaling/what-is-the-extracellular-matrix-and-the-basal-lamina/what-is-integrin/