Kindler Syndrome (KS) is an autosomal recessive disorder that results in skin blistering and potential development of squamous cell carcinoma, among other symptoms.[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, which is the **gap in knowledge** for this study.

The **objective** of this study is to identify the role of FERMT1 in mediating cell proliferation. *Danio rerio* will be the model organism used in this study because they are less expensive than mice, young zebrafish show a comparable phenotype to blistering in KS, and adult zebrafish can develop easily observable tumors. [2] The **hypothesis** for this study is that FERMT1 plays a large role in transcription and cell cycle regulation and disruption of this leads to uncontrolled cell proliferation. The **long term goal** of this study is to uncover the reasons for cancer development in older KS patients to uncover potential targets for preventative treatments for patients.

AIM1: Identify highly conserved residues of FERMT1 that maintain cell proliferation in adult fish
Rationale: Identifying and mutating highly conserved residues will produce comparable KS mutants in zebrafish. Since there is no specific domain mutated in KS patients, each domain will be mutated in separate lines of fish. Highly conserved residues are most likely to be involved in a highly conserved process like cell proliferation maintenance.
Approach: The sequence of FERMT1 in model organisms was aligned in MEGA using ClustalW. A glycine at site 44 (kindlin-2-N) and a leucine at site 398 (FERM) were identified to be highly conserved and within the domains and will be mutated via CRISPR-Cas9. The mutations will be to stop codons.
Hypothesis: This will result in the establishment of two mutant lines of zebrafish that model KS: kindlin-2-N mutants and FERM mutants. Mutant fish will show a ruptured fin phenotype in youth and develop cancer in adulthood.

AIM2: Identify differentially expressed genes in adult zebrafish mutants versus adolescents
Rationale: Differential transcription in FERMT1 mutants shown by single-cell RNA Seq analysis can provide further insight to FERMT1's influence on gene expression and cell proliferation. Single-cell RNA Seq allows for the study of one fish over time, ensuring changes are age related.
Approach: Single-cell RNA sequencing will be used to analyze transcription levels in dermal cells of young and adult zebrafish mutant's fins.
Hypothesis: Wild type fish will show consistent expression levels. Mutant fish will show decreased cell adhesion gene expression in youth. This expression level will recover in adulthood and transcription related genes will be expressed at higher levels than wild type.

AIM3: Identify protein interaction networks of FERMT1 in adult mutants versus adolescents
Rationale: FERMT1's protein interaction network show by STRING revealed a connection to transcription related proteins via CDC5L. A disturbance in this interaction could provide insight to FERMT1's role in transcription regulation and cell proliferation. BioID will allow the same fish to be studied *in vivo*, ensuring differences are age related.
Approach: Biotin will be added near the N-terminus of FERMT1 to ensure its binding is consistent across the mutants. Interactions will be observed in adult and young fish.
Hypothesis: Young FERM mutants will show large decreases in interactions with transcription proteins and adhesion proteins, with slight increases in adhesion proteins with age. Kindlin-2-N mutants will show a similar change over time with less overall interaction due to its greater truncation.

References

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