**Specific Aims**

Fanconi Anemia (FA) is a complex, autosomal recessive or X-linked genetic disorder that affects the ability of cells to effectively repair interstrand cross-link DNA damage [1,2]. FA patients have varying clinical manifestations from developmental delay to malformations of the eyes, ears, and thumbs as well as altered skin pigmentation, known as cafe au lait spots. There are three complexes involved in the FA DNA repair mechanism: the core complex, the ID complex, and the DNA repair complex [3]. Of particular interest is one of the core complex members, FANCL, an E3 ubiquitin ligase that plays a vital role in the signaling between the core complex and the ID complex. FANCL catalyzes the mono-ubiquitination of FANCD2, one of two proteins in the ID complex, and consists of two functional domains: the RING domain and the WD40 repeat domain. The RING domain is responsible for binding to ubiquitin and facilitating its transfer to FANCD2 [2], while the WD40 repeat domain is essential for the protein-protein interactions between FANCL and the other FA core complex proteins [5].

Although we know the structure and function of these domains, there are some questions remaining about how mutations in both the RING and WD40 domains lead to disease. Considering the importance of the gene, one crucial aspect of the disease that remains unclear is why mutations in the FANCL gene produce relatively mild and variable patient phenotypes. The goal of this research is to understand how mutations within the two different domains affect the function of the FANCL protein and to gain insight into why particular mutations have a greater effect on phenotype in FANCL-caused FA patients. By utilizing Zebrafish as a model organism, I will be able to characterize both phenotypic and proteomic changes that will provide a qualitative and quantitative approach to understanding the effect of FANCL mutations.

**Hypothesis** – Mutations occurring in the WD40 repeat domain that restrict the binding of FANCL to the other core complex proteins will have a greater effect on skin pigmentation alterations than those in the RING domain. This is due to a greater reduction of ubiquitination of FANCD2 because the core complex will not be able to assemble without proper binding to FANCL.

**Aim I** – Identify novel binding partners of Zebrafish FANCL that play a role in skin pigmentation.

Approach – Use STRING to identify Zebrafish binding partners and identify GO terms for each protein. Identify proteins with GO terms related to skin pigmentation and use the CRISPER/CAS9 system to knock out the proteins to verify if they play a role in skin pigmentation.

**Aim II** – Determine whether the WD40 or the RING domain plays a specific role in skin pigmentation.

Approach – Use ClustalW to identify conserved amino acid residues in both domains of the FANCL protein and then use the CRISPER/CAS9 system to induce site-specific mutations into highly conserved regions. Using these lines of transgenic Zebrafish, I will then screen for mutations that lead to skin pigmentation alterations.

**Aim III** – Determine the level of ubiquitination in each of the transgenic Zebrafish lines.

Approach – Utilize liquid chromatography tandem mass spectrometry with absolute quantification (AQUA) standards to quantify the level of FANCD2 ubiquitination. Perform analysis on the total level of ubiquitination in each transgenic line and compare the results to wild-type Zebrafish and the results obtained in Aim II.

Through these techniques, I will gain an understanding of how each domain plays a role in the overall function of the FANCL protein and in skin pigmentation. Overall, I will be able to identify which FANCL mutations lead to milder forms of Fanconi Anemia, with the hopes of uncovering the molecular mechanism of the disease.

References:

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