Cockayne Syndrome (CS) is a rare neurodegenerative disorder where patients exhibit sensitivity to light and premature aging. CS Type II, the congenital form of the disease, is caused by mutations in the ERCC6 gene [1]. ERCC6 regulates the transcription coupled nucleotide excision repair process. ERCC6 loss leads to an increased sensitivity to light. ERCC6 is thought to play a role in repairing DNA damage caused by oxidative damage, which causes aging [2], yet molecular role of ERCC6 in the premature aging phenotype remains unclear.

My **primary goal** is to determine how ERCC6 regulates DNA damage repair after oxidative stress associated with aging. I will use mice as a model organism because they have been shown to be an excellent model organism for studying oxidative damage and aging [3,4]. I **hypothesize** that ERCC6 has a DNA binding and lesion recognition role in the oxidative damage repair pathway, and ERCC6 loss leads to the aging phenotype in Cockayne Syndrome patients because oxidative damage cannot be regulated. My **long term goal** is to understand the role of ERCC6 in aging.

**Aim 1: Identify which ERCC6 protein domains are important for regulating oxidative stress during aging.**

**Hypothesis:** I hypothesize that because the ERCC6 helicase domain plays an integral role in DNA repair, a knockout of this domain will result in the aging phenotype after oxidative stress.

**Approach:** I will use SMART and Pfam to identify conserved protein domains in ERCC6 in mice. Using CRISPR/Cas9, I will create transgenic mice to mimic the human aging phenotypes. Next, I will induce oxidative stress. My controls will be: Wild type, WT with oxidative stress, and domain mutant without oxidative stress. I will determine the cellular response and aging phenotype in all lines by measuring levels of 8-hydroxydeoxyguanosine (8-OHdG), an oxidative derivative of guanosine [5,6].

**Rationale:** ERCC6 has two major protein domains: a SNF2\_N domain and HELICc domain. Deducing which domain helps mitigate oxidative damage levels will help clarify how the protein potentially affects oxidative damage and aging.

**Aim 2: Identify aging-associated genes that are differentially expressed after oxidative stress in ERCC6 domain loss.**

**Hypothesis:** The loss of the HELICc ERCC6 domain will lead to a downregulation of aging-associated genes.

**Approach:**  I will use RNA sequencing to analyze gene expression in the two ERCC6 mutants created in Aim1 compared with the Wild type control with and without oxidative stress. I will look for aging-associated genes using Gene Ontology that are downregulated after ERCC6 domain loss and oxidative stress.

**Rationale:** By observing differential gene expression between WT and ERCC6 domain knockouts in the presence of oxidative damage, I can determine clusters of aging-specific genes affected by ERCC6 domain loss.

**Aim 3:** **Confirm ERCC6 protein interactions under oxidative stress to infer its direct association with genes involved in oxidative damage regulation and therefore aging.**

**Hypothesis:** ERCC6 is a recruiter of proteins in excision repair pathways so it binds with other proteins in its interaction network with GO terms in the oxidative damage repair pathway when oxidative stress is induced.

**Approach:** Using STRING I will explore ERCC6’s interaction network. I will go through the results and look for genes with Gene Ontology terms involving oxidative damage regulation. I will use co-immunoprecipitation and Western blotting to test interactions between ERCC6 and proteins with aging/oxidative damage related GO terms, once in a Wild type mouse and once in WT with induced oxidative stress.

**Rationale:** By confirming the suspected interactions between ERCC6 and other proteins in its network associated with regulating oxidative damage, I can determine candidate genes that are directly impacted by ERCC6 loss as potential targets for therapeutic medicine in CS patients.

References

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