Familial Hemophagocytic Lymphohistiocytosis (fHLH) is an immune condition appertaining to overactivation and excessive formation of macrophages and T- lymphocytes. A subgroup of HLH, 'familial' or 'primary' HLH refers to the genetic variation of the disease in which a mutation in PRF1 is inherited [1]. Symptoms of fHLH result from decreased regulation of the macrophages, allowing excessive cytokine release. The cytokine causes hyperinflammation in multiple regions of the body, including the spleen, liver, brain, and bone marrow. Clinical diagnosis requires any five of the eight criteria: prolonged and high fever, splenomegaly, cytopenia (low blood cell counts), high triglycerides, hemophagocytosis in any tissues, low natural killer cell activity, excessive cytokine receptors, or hyperferritinemia in blood serum [2]. Despite being characteristically a criterion of diagnosis, high ferritin counts are not always a reliable predictive signal of fHLH [3]. The **role of iron homeostasis**, as partially directed by ferritin's iron storage, **in fHLH is unclear [1,4,5].** To study this interaction, **Dario Rerio** was chosen as the model organism. Zebrafish is a popular choice for modeling immunology systems due to its similar macrophage activity to humans [6]. Previous studies have also been completed with zebrafish to study the effects of ferric ions [7].

PRF1 mutation resulting in fHLH causes decreased pore formation for cytotoxicity. This relationship between this unregulated response and high ferritin be elucidated given the associated high mortality rate with hyperferritinemia [3]. My **objective** in this study is to identify gene expression variation associated with increased serum ferritin counts. I **hypothesize that** iron homeostasis in fHLH will result from the varying expression of ferritin-encoding genes. Clinical studies have determined that familial HLH has increased ferritin than that of non-genetic HLH [4]. My **long-term goal** is to deepen my understanding of the association of ferritin in autoimmune diseases.

Aim One: **Identify PRF1 protein domains involved in pore formation and associated homologs in Danio Rerio**

Rationale- Identification of protein domains in PRF1 and its conservation between Zebrafish and Homo sapiens will reveal possible mutation sites. **Approach**- Identified homologs will be inputted into InterPro scanning software to examine protein domains. Domains relating to cytotoxicity will be recorded and similarities between homologs will be analyzed. Multiple sequence alignment will then be performed between the two protein sequences to identify possible regions to induce mutation to cause fHLH like symptoms in the Zebrafish. CRISPR/Cas9 will then be used to induce mutation in region of choice. Blood serum will be analyzed in Zebrafish to confirm excess white blood cells is identified. **Hypothesis**- Pore formation domain is crucial for cytotoxic activity, so a mutation in this region would cause fHLH symptoms.

Aim Two: **Single-cell RNA sequencing and subsequent cluster analysis**

Rationale- Cluster data of PRF1 and iron homeostasis-associated genes will reveal any regulatory relationships between PRF1 mutation and ferritin. **Approach**- Zebrafish spleen tissue will be isolated using microscopy and a capillary pipette. The DNA of each cell will be amplified by PCR and sequenced using Illumina sequencing. Reverse transcription will be performed to result in an RNA library. Quality control will be performed, and gene expression will be quantified through RPKM. Principal components analysis will be performed to obtain gene expression clusters. Confirmation of hyperferritinemia symptoms in knockout zebrafish will be completed with fluorescence assay targeting ferric iron. **Hypothesis**- Ferritin encoding genes will be highly associated with PRF1 perforin.

Aim Three: **Analysis of proteome via metabolic labeling of knockout tissues**

Rationale- Labeling tissue experimental groups with arginine isotopes will reveal a fold increase/decrease in the system, revealing fHLH associations with ferritin. **Approach**- Tissue isolated in aim two will additionally be used for proteome analysis. PRF1 knockout tissue will be grown in medium with heavy arginine. Wild-type Dario Rerio tissue will be grown in a light arginine medium. After digestion with trypsin, mass spectroscopy will be completed on both experimental groups. Volcano plots will be generated for analysis of fold increase/decrease protein expression. **Hypothesis**- An increase in protein fold for ferritin will be observed in PRF1 perforin knockout tissues.

I would expect that this research would yield insight into the mechanisms of increased ferritin count in many clinical cases of fHLH, such as any additional genes or proteins that may be involved in this symptom. Understanding the relationship between mutated perforin pore formation and increased ferric iron could reveal possible treatment options for this symptom and/or the condition. With a 59% five-year probability of survival in diagnosed treated children [1], it is crucial to continue researching fHLH and striving for better treatment methods.

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