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Familial Hemophagocytic Lymphohistiocytosis (fHLH) is an immune condition caused by an overactivation and excessive formation of macrophages and T-lymphocytes. 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 (Zhang, 2021). Additional symptoms include prolonged and high fever, splenomegaly, and cytopenia or low blood cell counts (Janka et al., 2005). A mutation in the PRF1 gene is one of the causes of fHLH (Zhang, 2021). There are two recognized protein domains in this gene: the Membrane Attack Complex/ Perforin Domain as well as the Calcium dependent C2 Domain (Paysan-Lafosse et al. 2022). We will focus on the MACPF_2 domain for this discussion.

The MACPF_2 domain is the pore forming domain, where perforin subunits are encoded. The quaternary structure of perforin is the pore (Osinka et al., 2014). This protein localizes at the cellular membrane when the immune response is activated. The function of pore formation is involved in leukocyte mediated cytotoxicity (Paysan-Lafosse et al. 2022). When the recessive, loss- of- function mutation occurs as it does in fHLH, cytotoxins are unable to enter and degrade the infected cell. This leads to an increase in natural killer cell and T- cell activation, causing the excessive cytokine release (Osinka et al., 2014). The MACPF_2 domain is well conserved amongst

vertebrates, and is only found in organisms with an immune system (Paysan-Lafosse et al. 2022). A maximum likelihood phylogenetic tree for Perforin was generated using Mega11. Mammalian PRF1 is most closely related, but, given the well conserved nature of the protein domains, Danio Rerio is still a candidate for any proposed study of PRF1 mutation. (Tamura, Stecher, and Kumar 2021)

One possible symptom of fHLH is hyperferritinemia, or an excess of ferritin in the bloodstream (Zhang, 2021). Ferritin is a protein in which ferric iron is stored (Kernan et al., 2017). The role of iron homeostasis in fHLH is currently unclear. My hypothesis is that iron homeostasis in fHLH will result from varying expression of ferritin encoding genes [FTL FTH], and subsequent protein concentrations.

To test this relationship, Danio Rerio will be used as a model organism. Zebrafish is an excellent model due to its sensitivity to oxidative stress from ferric iron, as previous studies have also been completed with zebrafish to study the effects of ferric ions (Bohaud et al., 2021). Zebrafish is a popular choice for modeling immunology systems due to clear larval state. It also has a similar macrophage activity to humans (Ayaat et al., 2020). All of these reasons make zebrafish an ideal candidate for reverse genetics approaches. PRF1 has similar protein networks in both Homo Sapiens and Danio Rerio, with both being heavily involved in T- cell proliferation pathways as well as the innate immune response (Szklarczyk, 2023).

Aim one will involve the identification of protein domains essential for pore formation in Danio Rerio. As previously determined, the MACPF_2 domain is related to pore formation (Paysan-Lafosse et al. 2022). Multiple sequence alignment of this region will be performed between the Danio Rerio and Homo Sapiens to identify possible regions to induce mutation to cause fHLH like symptoms in the Zebrafish. One identified mutation in Homo Sapiens that causes fHLH is the Glycine to Alanine mutation at the 445th location (Zhang 2011). The sequence alignment will be used to confirm that this region of interest has no gaps in Danio Rerio. CRISPR/Cas9 will then be used to induce mutation in the region of choice. Blood serum will be analyzed in Zebrafish to confirm excess white blood cells are identified. We hypothesize that a mutation in this region would cause fHLH symptoms since the pore formation domain is crucial for cytotoxic activity.

Aim two will work to identify variations in gene expression in PRF1 and Iron homeostasis genes. Zebrafish spleen tissue of the induced knockouts as well as the control fish 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 Principal components analysis will be performed to obtain gene expression clusters. Cluster data of PRF1 and iron homeostasis-associated genes will reveal any regulatory relationships between PRF1 mutation and ferritin (Hwang et al., 2018). Confirmation of

hyperferritinemia symptoms in knockout zebrafish will be completed with fluorescence assay targeting ferric iron. I hypothesize that Ferritin encoding genes will be highly associated with PRF1 perforin (Ayaat et al., 2020).

Aim three contains an analysis of the proteome via metabolic labeling of control and knockout tissues. To approach this aim, tissue isolated from knockout zebrafish will be grown in medium with heavy arginine. Wild-type Danio Rerio tissue will be grown in a light arginine medium. After digestion with trypsin, mass spectroscopy will be completed on both experimental groups (Ong et al., 2007). Volcano plots will be generated for analysis of fold increase/decrease protein expression (Choi et al., 2022). Labeling tissue experimental groups with arginine isotopes will reveal a fold increase or decrease in the system, which would reveal any fHLH associations with ferritin. The fluorescence assay targeting ferric iron will once again be used as confirmation. I hypothesis that an increase in protein fold increase for ferritin will be observed in PRF1 performing knockout tissues.

In conclusion, fHLH is an autoimmune condition where a PRF1 mutation can cause damage to the immune response. The relationship between this condition and high iron homeostasis is unknown. I explored Danio Rerio as a model organism for PRF1 mutation and how it can be used to elucidate this relationship. I proposed multiple genomics/proteomics approaches to exploring the relationship between these pathways. Future research should be targeted towards relieving symptoms of fHLH

such as hyperferritinemia. This could be accomplished using a small molecule assay to discover any drugs or small molecules that may decrease said symptoms.

References

Ayaat T. Hassan, et al. “The Neurophysiological Effects of Iron in Early Life Stages of Zebrafish.” Environmental Pollution, Elsevier, 18 Sept. 2020, www.sciencedirect.com/science/article/pii/S0269749120363132#abs0015.

Bohaud, Candice, et al. “The Role of Macrophages during Zebrafish Injury and Tissue Regeneration under Infectious and Non-Infectious Conditions.” Frontiers, Frontiers, 2 July 2021, www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.707824/full.\

Choi, S., Engelke, R., Goswami, N., & Schmidt, F. (2022). Proteomic profiling of metformin effects in 3t3-L1 adipocytes by silac-based quantification. *PROTEOMICS, 22*(11–12). https://doi.org/10.1002/pmic.202100196

Glen Stecher, Koichiro Tamura, and Sudhir Kumar (2020) Molecular Evolutionary Genetics Analysis (MEGA) for macOS. Molecular Biology and Evolution 37:1237-1239

(Publication PDF available at https://www.megasoftware.net/citations)

Hwang B, Lee JH, Bang D. Single-cell RNA sequencing technologies and bioinformatics pipelines. Exp Mol Med. 2018 Aug 7;50(8):1-14. doi: 10.1038/s12276-018-0071-8. Erratum in: Exp Mol Med. 2021 May;53(5):1005. PMID: 30089861; PMCID: PMC6082860.

Janka, Gritta, and Udo zur Stadt. “Familial and Acquired Hemophagocytic Lymphohistiocytosis.” American Society of Hematology, American Society of Hematology, 1 Jan. 2005, ashpublications.org/hematology/article/2005/1/82/19290/Familial-and-Acquired-Hemophagocytic.

Kernan KF, Carcillo JA. Hyperferritinemia and inflammation. Int Immunol. 2017 Nov 1;29(9):401-409. doi: 10.1093/intimm/dxx031. PMID: 28541437; PMCID: PMC5890889.

Murtha, Jill, et al. “Hematologic and Serum Biochemical Values for Zebrafish (Danio Rerio).” Ingenta Connect, 2003,

www.ingentaconnect.com/content/aalas/cm/2003/00000053/00000001/art00005?crawler=true&mimetype=application/pdf.

Ong, Shao-En, and Matthias Mann. “A Practical Recipe for Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC).” *Nature News*, Nature Publishing Group, 11 Jan. 2007, www.nature.com/articles/nprot.2006.427.

Osińska, Iwona, et al. “Perforin: An Important Player in Immune Response.” Central-European Journal of Immunology, U.S. National Library of Medicine, 2014, www.ncbi.nlm.nih.gov/pmc/articles/PMC4439970/.

Paysan-Lafosse T, Blum M, Chuguransky S, Grego T, Pinto BL, Salazar GA, Bileschi ML, Bork P, Bridge A, Colwell L, Gough J, Haft DH, Letunić I, Marchler-Bauer A, Mi H, Natale DA, Orengo CA, Pandurangan AP, Rivoire C, Sigrist CJA, Sillitoe I, Thanki N, Thomas PD, Tosatto SCE, Wu CH, Bateman A. InterPro in 2022. Nucleic Acids Research, Nov 2022, (doi: 10.1093/nar/gkac993)

Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Annika GL, Fang

T, Doncheva NT, Pyysalo S, Bork P‡, Jensen LJ‡, von Mering C‡.The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023 Jan 6;51(D1):D638-646

Zhang, K., et al. “Hypomorphic Mutations in PRF1, MUNC13-4, and STXBP2 Are Associated with Adult-Onset Familial HLH.” *Ash Publications*, 2011, ashpublications.org/blood/article/118/22/5794/29198/Hypomorphic-mutations-in-PRF1-MUNC13-4-and-STXBP2.

Zhang, Kejian. “Familial Hemophagocytic Lymphohistiocytosis.” GeneReviews® [Internet]., U.S. National Library of Medicine, 30 Sept. 2021, www.ncbi.nlm.nih.gov/books/NBK1444/.