Optic atrophy type 1 is an autosomal dominant genetic disorder caused by mutations within the OPA1 gene [1]. The OPA1 gene can be found mainly in the retinal ganglion cells [2]. Mutations in the OPA1 gene disrupt normal protein production and lead to altered mitochondrial structure [3]. This mitochondrial dysfunction triggers apoptosis in optic nerve cells, impairs synaptic transport, and ultimately results in optic atrophy type 1 [5]. Based on recent studies, it is known that optic atrophy type 1 patients suffer from blindness, blocked vision, or color vision deficiency [4]. *However, the role of OPA1 in the maintenance of mitochondria during retinal development remains unknown.*

My **objective** is to determine the role of OPA1 gene in mitochondria maintenance during retinal development. Danio rerio (zebrafish) will be used as the model organism to conduct the experiments since the zebrafish have rapid, transparent embryonic development, and exhibit similar retinal ganglion cells to humans [7]. The dysfunctions caused by the mutations can be assessed by examining the structures of the optic nerve and the mitochondria of the zebrafish models. I **hypothesize** that OPA1 functions during embryonic stages to maintain mitochondria structure in the retina. My **long-term goal** is to conduct more advanced research regarding optic atrophy type 1 disease and find novel protein interactions with the OPA1 gene.

Aim 1: Identify gene domains in OPA1 that are crucial for mitochondria development in the retina.
Hypothesis: Deletion or deactivation of the guanine-binding domain will rescue the disease-like phenotypes.
Rationale: Determining the function of each gene domain of OPA1 can identify which domain is responsible for the mitochondria development.
Approach: Domain **analysis** will be conducted to analyze the human and zebrafish OPA1 gene to determine major functional domains using SMART. Two domains were identified: the guanine-binding domain and the GTPase C-terminal. CRISPR/Cas9 will be employed to knock out the two identified domains, the guanine-binding domain will be knocked out in the first mutant while the GTPase C-Terminal will be knocked out in the other mutant. Both wildtype and the two mutant zebrafish will undergo chemical screens to validate the phenotypes. The designated time for chemical screens would be 24 hours, 48 hours, and 72 hours post-hatching. Based on recent studies, the guanine-binding domain contains three conserved amino acids identified by MEGA: R290Q, G300E, and L384F [6]. The three identified amino acids will be cut off to inactivate the guanine-binding domain, chemical screens will then be conducted for phenotypic analysis.

Aim 2: Identify chemical compounds that can rescue the phenotypes of optic atrophy type 1.
Hypothesis: Different chemical compounds rescue the shape of mitochondria to different extents, some of them are suitable for drug discovery in clinical use.
Rationale: Chemical compounds can interact with the amino acids and affect the function of the gene. By identifying new chemical compounds, altered mitochondria structures can be rescued and develop clinical treatments.
Approach: Chemical compounds will be selected from the Stanford High-Throughput Screening Knowledge Center Library (HTSKC) since it contains chemicals suitable for high-throughput chemical screening and can rescue the phenotypes. The selected chemicals will be injected into the embryos of newly bred wild-type and mutant zebrafish. The wild-type and mutant embryos will be assayed and stained for chemical screens. By analyzing the results, the compound that works the best in rescuing the phenotypes can be identified.

Aim 3: Quantify proteins in wildtype and mutant zebrafish that associate with mitochondria maintenance during development.
Hypothesis: Mutant OPA1 zebrafish with altered mitochondria structures will exhibit elevated protein abundance levels.
Rationale: Quantifying proteins could identify proteins that are affected by the mutation of OPA1 in mitochondria during the mitochondria development stage, knockout of proteins with elevated abundance levels can determine if up-regulated genes can mitigate the observed phenotypes.
Approach: Tissue cells will be extracted from both wild-type and mutant zebrafish at 24 hours, 48 hours, and 72 hours post-hatching. iTRAQ will be applied to separate and digest proteins into peptides, which will then be labeled with isobaric tags and analyzed with mass spectrometry to measure protein abundance levels. By comparing the results from the wild-type and mutant zebrafish, proteins that display elevated abundance levels in mutants will be targeted for knockout using CRISPR/Cas9. The structure of mitochondria and optic nerve of both wild-type and mutant zebrafish will be examined for changes.

In conclusion, mutations in the OPA1 gene can induce mitochondria dysfunction and cause optic atrophy type 1. Potential chemical compounds can be identified for rescuing the shape of mitochondria back to normal. Also, OPA1 protein interactions may help in identifying novel therapeutical treatments. In the future, new binding proteins can be identified using BioID.

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