Randy Maysaud – EN.585.685.81 – Module 7 Case Study – Models in Neurobiology

Background:

Parkinson's disease is a nervous system disorder that negatively affects the patient's movement control. The symptoms may start with a small tremor and gradually grow to larger tremors or even stiffness of movement.[1] One of the suggestive causes of Parkinson's Disease is by the cell death of dopamine neurons, allowing for dangerously low levels of dopamine. In Parkinson's Disease the death of dopamine neurons can be caused by an abnormal protein accumulation of a protein named alpha-synculein (α-Syn).[2] In Parkinson's Disease there is a mutated version of the gene SNCA that provides the instruction of creating the α-Syn protein, resulting in the abnormal amount of the protein.[3]

The fish Nothobranchius Furzeri (N. Furzeri) is a rapid aging short lived animal. It is very difficult to get an animal model to exhibit characteristics of Parkinson's Disease, however the N. Furzeri naturally exhibits dopaminergic neuron degeneration like Parkinson's Disease. N. Furzeri also has a progression of the α-Syn protein, making the fish a natural model for Parkinson's Disease.[4]

Telomerase is an enzyme is that will maintain the length of telomeres by adding the repetitive sequence at the end of chromosomes.5 The addition of telomerase in Parkinson's Disease has shown promising effects in research models, however once they notice the disease and inject the telomerase it is too late. Around 30% of the dopaminergic neurons are dead. TERT gene provides the instructions for creating a component of the telomerase.[6]

Aim:

What affect will an increase in telomerase have on the life span and dopaminergic neurons of the N. Furzeri fish?

Research Plan:

<u>Overview</u>

In this research plan we will be modifying the telomerase gene in N. Furzeri fish to increase the length of telomeres using CRISPR. We will have two groups of N. Furzeri fish, group A will not receive the gene therapy and group B will receive the telomerase therapy. After 10 weeks we will sacrifice half of both groups and analyze the brain using CLARITY method. The other half will be kept alive to track their lifespan. Group A and B will consist of around 20 N. Furzeri fish for each group.

<u>Procedure</u>

1. Use CRISPR to genetically modify the telomerase gene in group B.
    a. To perform a gene knockout, CRISPR will us the sgRNA to create a ribonucleoprotein and the CAS9 protein will lead the CRISPR to the telomerase gene, named TERT. The CRISPR will create a double stranded break on the

TERT gene and remove it. Using CRISPR we will add a mutated TERT gene that increase the output of telomerase increasing the length of telomeres.
   b. This genetic modification will be made to the embryo of the fish, before the dopaminergic cells start to die.

2. Wait for 10 weeks and euthanize half the surviving fish in each group to use the clear method on the N. Furzeri fish. The other half will be used to view the changes in life expectancy if any.

3. View the dopaminergic neurons in the euthanized fish in group A and B by using the CLARITY method. This will allow us to compare the results.[7]

   a. The brain tissues will be fixed with formaldehyde and acrylamide monomers, this will allow links between the molecules and monomers. The monomers will then polymerize into a hydrogel mesh.
   b. The lipids in the tissue will be removed by an SDS detergent.
   c. Stain the clear tissue within the midbrain which has the dopaminergic neurons as well as the receptors.
   d. The labeled tissues will be put in RI-matching solution
   e. The tissues will become transparent, and we can use the microscopes to view the brain

4. Record the lifetime of each fish in both group A and B.

Conclusion:

There has already been some success with this experiment, however it has never been tried on a N. Furzeri fish. I believe that this research project will have a positive output and may extend the life of the fish, however I fear the chance of creating a cancer. Modifying the telomeres length is dangerous and can cause harm, I am interest to view what side effects this research project may bring. The usage of clarity will allow us to view many aspects of the brain and can help find discoveries that might have been overlooked. This research project is specific to aging, because Parkinson's Disease is a disease that worsens as the brain ages, therefore using the N. Fruzeri fish is perfect, because of its similarity in dopaminergic neuron degeneration and its short lifespan.

References:

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