The Pathogenesis of Friedreich's Ataxia Using Proteomic Analysis of DRG

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Abstract

Friedreich's Ataxia is the only known genetic disorder that requires the inheritance of two copies of the abnormal FXN gene to cause the disease. Both parents must carry the defective gene in order for the individual to get this disease. FA is a guanine-adenine-adenine trinucleotide repeat in the frataxine gene. The purpose of this experiment was to prove that there is a increase in the response of the dorsal root ganglion to the antibody from patients with Friedreich's Ataxia compared to patients without Friedreich's Ataxia. To prove this, completed immunohistochemistry with tissue lysates. Immunohistochemistry itself has many steps to it including tissue fixation, antigen retrieval, and the labeling of the slides. The last step of immunohistochemistry, or the labeling of the slides, will lead us to the next step in our experiment which the double label immunoflourescence procedure. In this procedure, we put anti-mouse IgG and KIT pY936 on the positively charged glass slides. Then we were able to look at these slides under a confocal microscope. From this, we were able to see that more nuclei were responsive in the patients that had Friedreich's Ataxia compare to the patients without this disease.

Introduction

Friedreich's Ataxia is a rare genetic disorder which severely affects the central nervous system and causes difficulty in movement. FA is a disease in which low levels of Frataxin lead to nerve fibers in your spinal cord and peripheral nerves becoming thinner. Peripheral nerves carry information from the brain to an individual's body. Frataxin works as a "collector" of iron and sulfur throughout the body. Another part of the body that degenerates is the cerebellum in the brain, although the damage to the cerebellum is less significance than the damage to the nervous system. This is because the disease is specifically designed to attack the nervous system- not the brain.

The cerebellum plays an important role in the human body as it plays a vital role in an individual's ability to coordinate balance and movement. Friedreich's Ataxia degeneration begins in an individual's childhood which leads to impaired muscle coordination which will continue to worsen over time. The life expectancy of someone with this disease is early thirties, although some can live into their sixties. Trouble walking, fatigue, slowed speech, and changes in sensations tend to begin in late childhood, and are all symptoms of this disease. In addition, Friedreich's Ataxia may also cause heart disease and diabetes.There is currently no known cure for the disease.

This experiment aims to discover a correlation of the upregulations of proteins in lysates of dorsal root ganglion in patients with Friedreich's Ataxia. Dorsal root ganglion is a collection

of cell bodies of sensory neurons. Sensory neurons are neurons that carry messages from an individual's senses to the brain. The dorsal root ganglion was used because they are exposed to the frataxin deficiency in Friedreich's Ataxia.

Materials and Methods

Materials:

Materials used within this experiment included tissue lysates of DRG previously prepared from patients with FA as well as tissue lysates of DRG previously prepared from patients without FA. Other materials included a confocal microscope, which served as an aid for microscopic examination on the slides themselves. Ethanol, methanol, xylene, acetone, paraformaldehyde, and perfusion fixation were used as well.

Methods:

The experiment began by sorting through many antibodies using the antibody microarray. Microarray is a type of technology that analyzes many genes and antigens at the same time. The DNA, RNA, or protein is placed on a glass slide. Next, they are labeled with different fluorescent dyes. Once scanned, we are able to analyze our data on these nucleic acids and proteins.

The second step in completing this experiment was to pull out the tissue lysates of dorsal root ganglion from patients with and without Friedreich's Ataxia out of our freezer. The tissue lysates of dorsal root ganglion from patients without Friedreich's Ataxia were to be used as a

control group. The tissue lysates used in this experiment were previously prepared for use in a separate experiment. Tissue lysates are mixtures of substances that are made by the disintegration of the cell membrane. The tissue lysates were used to see the inter-cellular (DNA, RNA, proteins, and organelles from a cell) materials that are released when tissue lysates are made. To make the tissue lysates, frozen connective tissue of dorsal root ganglion must be dissected. Once this is done, the frozen tissue is put in a tube of lysis buffer. This buffer is made up of 30 ml NaCl (sodium chloride), 100 ml of 10% NP-40 (a type of surfactant which is used to reduce the surface tension of the buffer), 1 M Tris (a buffer made of sodium chloride, calcium chloride, sodium azide, hydroxymethyl, and 200 ml of deionized water), and 820 ml of H_2O. The buffer is stored at 4°C until it is ready to be used. Once the tissue is in the tube of lysis buffer, it sits on ice for 5 minutes. Then, the tubes of tissue lysates go in the centrifuge, a machine that rotates at a rapid rate to separate the fluids that are of different densities, for 5 minutes at 500*g*. When the lysates have come out of the centrifuge, they must be placed in 1ml of wash buffer. After this is done, the lysates are mixed with 50% iodixanol (a medication that helps diagnose and find problems in the brain). The lysates are centrifuged at 10,000g for 30 minutes. Once this step is completed, the lysates must be placed in the wash buffer once more. After this is done, the lysates are ready to use.

In this experiment, immunohistochemistry was completed with the tissue lysates. Immunohistochemistry is a tool that helps identify abnormal cells. It uses antibodies in order to

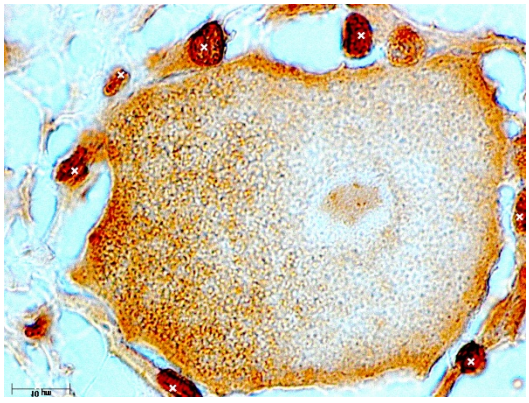
detect antigens, or proteins, that are specific to that abnormal cell. In this case, it was helping us find the proteins that are specific to Friedreich's Ataxia within the specific connective tissue that is used from the dorsal root ganglion. The purpose of fixation in immunohistochemistry is to keep everything in its place. Tissue fixation is crucial to the process of immunohistochemistry because it maintains the structure of the tissue. It also allows for the proteins to be detected by the antibodies. The procedure also requires antigen retrieval which is done so that there are more proteins that can be detected. It lowers the alteration of chemicals that are used.

Labeling of the antibodies is the last step to get the results of the procedure of immunohistochemistry. First, we mounted our tissue onto slides that were positively charged. They air dried for 30 minutes under a machine with direct airflow. As the slides air dried, we began to prepare the fixative. This fixative is made of paraformaldehyde, acetone, and a 1:1 solution of acetone : methanol. The slides soaked in this fixative for 15 minutes and then we rinsed them four times in phosphate buffered saline. Next, they airdried for 30 minutes under the machine with direct airflow.

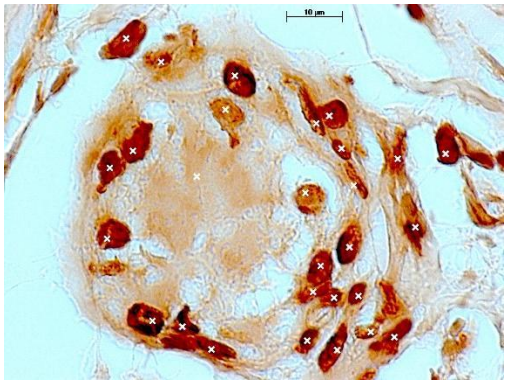
Antigen retrieval allows for an antibody to gain access to the target protein within the tissue itself. The heat induced method for antigen retrieval was used in this experiment. This method was used because it has a shorter incubation time, and the parameters of the section are more definable. The last step was to label the slides.

For this experiment, we used the double label immunofluorescence and confocal microscopy method. The purpose of doing this was because it is more visible when using this method. In this method, two or more different proteins can be looked at in the same sample. We used the double label immunofluorescence and confocal microscopy because the target can be easily detected from the second antibody. For this, we had to use one monoclonal antibody and one polyclonal antibody in order to see vivid results in our staining. We used the anti-mouse IgG monoclonal and Kit pY936 polyclonal. After letting these antibodies sit on the slides, we applied a layer of Triton X-100. The purpose of this was to get a thin layer on top of the slides in order to see results more vividly under the confocal microscope.

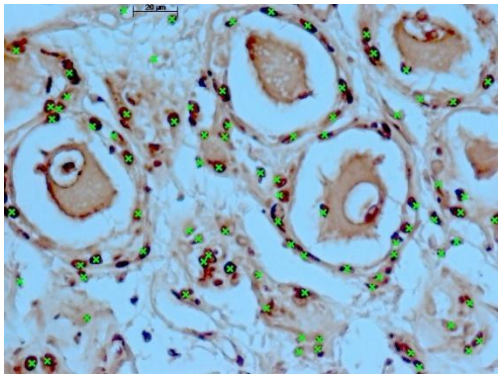
Results



Normal dorsal root ganglion with the KIT pY936 {Figure 1}

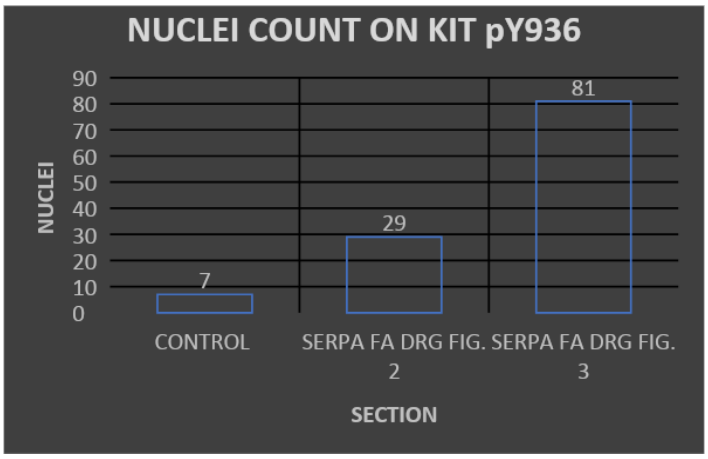


Serpa FA DRG with KIT pY936 Sample 1 {Fig. 2}



Serpa FA DRG with KIT pY936 Sample 2 {Fig. 3}

NAME OF SECTION	CONTROL	SERPA FA DRG FIG. 2	SERPA FA DRG FIG. 3
NUCLEI COUNT	7	29	81



We did not receive results from the microarray because the sensitivity was low. The microarray approach also did not work because it did not identify variants and the specifics of the genes.

Figures two and three show that the nuclei are more abundant in Friedreich's Ataxia than it in the control group. Figures one, two, and three show an overlap between the labels. This is

why some of the areas are darker than others. “Y” stands for the amino acid tyrosine, and 936 is the position that the tyrosine is at in the chain of amino acids. The KIT pY936 antibody grows invasive cells which kill nerve cells in the dorsal root ganglion. The green crosses in figures one, two, and three show the count of the nuclei around each nerve cell. It is visible in figure one that only a few nuclei react to KIT pY936 in the normal control group.

Discussion

This experiment proved my hypothesis correct, in that there is an upregulation, or an increased response to a substance, in patients with Friedreich’s Ataxia. The data is important because it shows that the proteins in the control group are not as responsive to KIT pY936 as the proteins of the patients with Friedreich’s Ataxia are. Further research would need to be necessary in order to answer the questions raised by the results of this experiment. At this time, we are not completely sure what this will mean for the disease itself- Friedreich’s Ataxia. Future research may be done to answer the following questions.

- What does this mean for Friedreich’s Ataxia?
- What does the upregulation prove?
- Could the finding of the upregulation in FA lead us to a cure for this disease?

References (Literature Cited)

U.S. Department of Health and Human Services. (n.d.). *Friedreich ataxia*. National Institute of Neurological Disorders and Stroke. Retrieved February 12, 2023, from https://www.ninds.nih.gov/health-information/disorders/friedreich-ataxia#:~:text=Friedreich ataxia

Gérard, C., Archambault, A. F., Bouchard, C., & Tremblay, J. P. (2023). A promising mouse model for Friedreich Ataxia progressing like human patients. *Behavioural Brain Research*, *436*, 114107. https://doi.org/10.1016/j.bbr.2022.114107

Koeppen, A. H. (2011). Friedreich's ataxia: Pathology, pathogenesis, and molecular genetics. *Journal of the Neurological Sciences*, *303*(1-2), 1–12. https://doi.org/10.1016/j.jns.2011.01.010