Biomedical Research: Inflammation and Insulin Resistance & Characterization of Lysosomal Storage Disease in a Mouse Model

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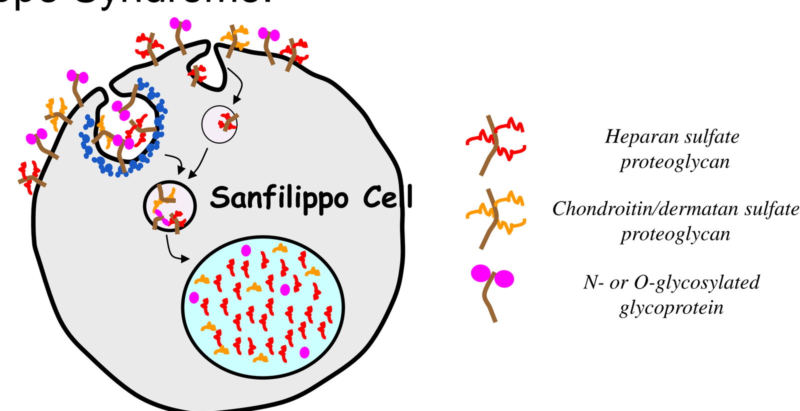
Introduction – Two different topics in Biomedical Research

Inflammation and Insulin Resistance: According to the American Diabetes Association, diabetes is a disease that in 2007 affected 23.6 million children and adults in the United States (7.8% of the population). In healthy patients, an increase in blood glucose results in the release of the hormone insulin which signals to many different cell types, such as macrophages, hepatocytes, myocytes and adipocytes to increase their uptake of this sugar. A patient affected with diabetes either does not produce enough insulin (Type 1 Diabetes), or does not respond to the insulin (Type 2). Unfortunately, with regards to Type 2 diabetes, it is still unclear how these cells become insulin insensitive. Recent data has suggested that a high concentration of free fatty acids may be signaling through a receptor called TLR4 (which usually binds to the bacterial sugar lipopolysacchiride or LPS) and that this signaling is resulting in a failure to respond to insulin. Understanding if this is the case and how this signaling results in insulin insensitivity is critical to understanding this disease and identifying novel targets for therapy.

Characterization of Lysosomal Storage Disease: The research laboratory is focused on glycobiology and the treatment and understanding of Mucopolysaccharidosis (MPS). Mucopolysaccharidosis are group of diseases in which glycosaminoglycans (GAGs), such as heparan sulfate, accumulate in the lysosomes due to enzyme deficiency. Stored heparan sulfate (HS) in the lysosomes causes progressive damage throughout the body, including the heart, bones, joints, respiratory system, and central nervous system. Patients with a subtype of Human Sanfilippo Syndrome, MPS IIIa, are deficient for the enzyme Sulfamidase which is essential to catabolyze heparan sulfate. The incomplete brake down of Heparan Sulfate results in the storage of carbohydrate fragments within cells causing progressive damage. My specific aim was to characterize glycosaminglycan storage in MPS IIIa mice to determine if they are a good model for Human Sanfilippo Syndrome.

This is a model for storage of Heparan Sulfate and other metabolites in a Human Sanfilippo Cell



Method

Inflammation and Insulin Resistance: The purpose of these experiments is to confirm that fatty acids phosphorylate BMI-1. If this is shown, then we will seek to identify the signaling molecules that result in BMI1 phosphorylation as well as the epigenetic and physiological changes that occur downstream.

This is done by Western Blotting lysates from macrophages and fibroblasts that have been treated with LPS, BSA (as a control) and Palmitic Acid (fatty acid). Apparently though, there are more things that interact with BMI1. Polymerase Chain Reaction is also used to multiply the BMI1 insert so it can be used to transform bacterial DNA. (Flow Chart)

By adding radioactive phosphate groups, it will be possible to determine where exactly BMI1 is being phosphorylated. However, this methodology is going to take a long time.

Characterization of Lysosomal Storage Disease:

Purification of GAG

Liver and brain were taken from wild type or MPS IIIa mice. The organs were homogenized and the lysosomes were purified using a Percoll density gradient. GAGs were purified from different fractions using anion exchange chromatography (DEAE). Then the samples were desalted using gel filtration chromatography (PD-10 columns).

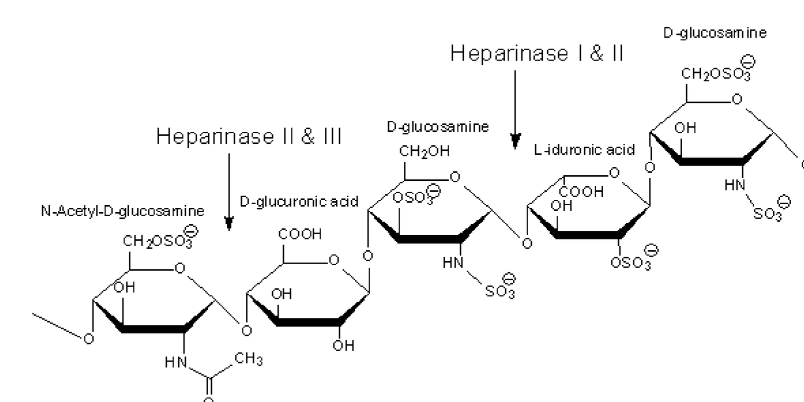
Purification of Enzyme

The production of the enzymes Heparinase I, II, and III (Hep I, II, and III) are useful to quantify the amount of HS. Hep I, II, III are produced as recombinant proteins in E. Coli. After lysing the cells, proteins were purified using affinity chromatography (Nickel column) followed by gel filtration chromatography.

Fast Protein Liquid Chromatography (FPLC) was used to purify the enzymes using affinity (Nickel column) and gel filtration (desalting column) chromatography.

Quantification of GAG

GAGs were subsequently treated with a mixture of heparinases I, II and III or chondroitinase ABC to specifically cleave heparan sulfate or chondroitin/dermatan sulfate, respectively. Because these enzymes are lyases they generated a double bond which can be detected by UV radiation (232 nm).



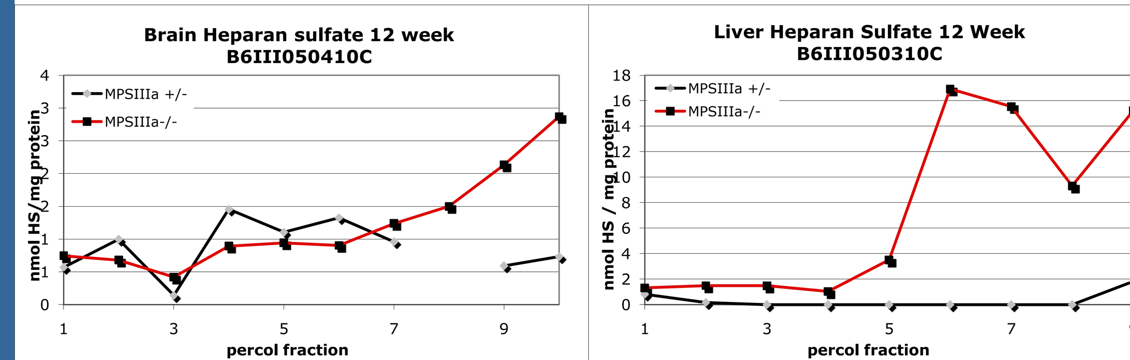
This is a model of a chain of Heparan Sulfate and the sites of activity of the enzymes Heparinsase I, II, and III.

Results

Inflammation and Insulin Resistance: Results have not been acquired yet because clear data showing the phosphorylation of BMI1 is needed first. However the expected results will be that BMI1 is indeed being phosphorylated by fatty acids, modulating its activity and changing the expression levels of a wide variety of genes. It will result in the confirmation that chronic phosphorylation of BMI1 may result in semi-permanent epigenetic changes which may then result in insulin insensitivity.

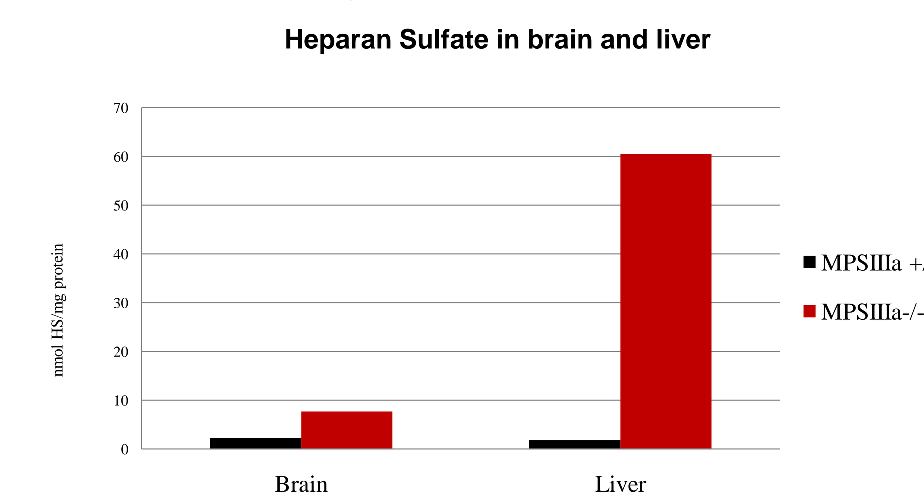
Characterization of Lysosomal Storage Disease: Increased Heparan Sulfate in the lysosomes of MPS IIIa

Fractions from the Percoll gradient were analyzed for Heparan Sulfate and Chondroitin/Dermatan Sulfate content as described in the methods. MPS IIIa mice showed increased HS levels in dense fractions corresponding to the lysosomes.



This graph represents the amount of Heparan Sulfate stored in the liver and in the brain of MPSIIIa mice and wild type mouse at 12 weeks of age showing an increase of storage.

The following graph shows the accumulation of Heparan Sulfate in MPS IIIa and wild type mice. It is notorious the difference of stored Heparan Sulfate in sick cells and wild type cells.



This graph represents the primary storage of Heparan Sulfate in brain and liver of MPS IIIa 12 weeks old mice.

Conclusion

Inflammation and Insulin Resistance: This project is designed to be a long term project which I will follow closely once the internship is over. The lab experience acquired thanks to this job is unforgettable. Other than working in such interesting topic, I had the opportunity to work with a very unique mentor and have a great time with everybody in Dr. Karin's lab.

Characterization of Lysosomal Storage Disease: The production and purification of Hep I, II, III was essential to quantify the amount of heparan sulfate in lysosomes that were purified from liver and brain of MPS IIIa mice and its control. Results show that Heparan Sulfate storage is significantly increased in both organs of MPS IIIa mice at 12 weeks of age. These results demonstrate that MPS IIIa mice can be used as a model to test both treatment strategies developed by the lab (Substrate reduction therapy and Enzyme Replacement Therapy). It is also thought that accumulation of HS can inhibit other lysosomal enzymes causing accumulation of a second metabolite, Chondroitin/Dermatan Sulfate and increase the severity of the disease. More experiments are needed to be done to test these hypotheses.

Acknowledgements

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