Probing GM1 Gangliosidosis to determine a biomarker for neurodegeneration.

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Neurodegenerative diseases affect approximately 50 million Americans each year, this includes Alzheimer's, Parkinson's, Huntington's disease and lysosomal storage diseases (LSDs). One type of storage LSD is GM1 Gangliosidosis. GM1 Gangliosidosis can occur in three types: Type 1 infantile onset, Type 2 juvenile onset, and Type 3 adult onset. Type 1 GM1 Gangliosidosis is the most devastating form, affecting 1 in 100,000-200,000 live births with patients not living past early childhood. In this and other neurodegenerative diseases, lysosomal hydrolases are upregulated, but the relationship between this upregulation and neurodegeneration is unknown. There are three well understood phases of neurodegeneration: increased autophagy, mitochondrial dysfunction, and neuroinflammation. Autophagy is the cellular process in which cells break down and recycle unwanted cytoplasmic material. In the cell, a membrane forms around cytoplasmic material forming an autophagosome. This then fuses with a lysosome to form an autolysosome. The lysosomal enzymes breakdown the cytoplasmic material stored in the autophagosome. When autophagy is impaired, the lysosome is unable to fuse with the autophagosome, leading to accumulation of toxic material in the cell. This project aims to determine the correlation between autophagosomal failure and lysosomal hydrolase upregulation. If the point of enzyme upregulation is determined, then the progression of this disease, and other neurodegenerative diseases, will be able to be determined, and in turn, can be treated in a more timely and patient specific manner.

Immortalized skin fibroblast lines isolated from felines, GM1SV3 (GM1 Gangliosidosis-affected felines) and NSV3 (normal felines), were grown to confluence in DMEM supplemented with 10% FBS and 1X penicillin-streptomycin at 37°C and 5% CO_2. After trypsinization, cells were passaged into chamber slides at a seeding density of 187,000 cells per mL and allowed to adhere. CellLight Lysosome-GFP was then added to the wells and incubated to stain lysosomes. After incubation, the cells were fixed to the slide, permeabilized, and blocked using donkey serum. Primary antibody, anti-LC3B, was added at 1:300 dilutions to stain autophagosomes and allowed to incubate overnight at 4°C. Cells were then washed and 2 drops of secondary antibody were added and allowed to incubate for 30 minutes at room temperature, protected from light. 4′,6-diamidino-2-phenylindole (DAPI) counterstain was then added and the slides were sealed. Cells were simultaneously passaged into 6 well plates at a seeding density of 100,000 cells per mL and grown to 100% confluence. Cells were scraped off of the surface of the wells and cell lysates were collected for enzyme activity analysis. Activities of hexosaminidase A, hexosaminidase T, mannosidase, and β-galactosidase were determined using 4-methylumbelifferone (4MU) substrates of each respective enzyme using previously established methods.

Immunofluorescence images of GM1SV3 showed higher green intensity when compared to NSV3. This indicates increased numbers of lysosomes present in GM1SV3. Red intensity is also enhanced in GM1SV3 cells, indicating an increased number of autophagosomes. This supports the idea that autophagy is impaired in the diseased cell line. The color yellow is also faintly present in NSV3, indicating colocalization of lysosomes and autophagosomes, meaning that the two are fusing during autophagy. Enzyme assay results showed consistent increased activity of total hexosaminidase in GM1SV3, showing a higher fold normal than other enzymes. This shows that there could be the upregulation of lysosomal enzymes hexosaminidase A, hexosaminidase B, or both. Assays also showed potential mannosidase upregulation, although yet statistically insignificant. The enzyme assays confirmed that β-galactosidase was greatly decreased in GM1SV3 cells when compared to normal type cells. In order to determine if

impaired autophagy leads to enzyme upregulation, NSV3 cells were starved. Starving cells induces impaired autophagy. Cells were starved for 2, 4, 6, 12, 24, and 48 hours. To confirm autophagy was impaired, starved NSV3 cells were imaged and colocalization, or more notably lack thereof, determined. Immunofluorescence images of starved NSV3 looked increasing similar to GM1SV3 as starvation times are increased, with 24 and 48 hour starvation leading to images with even more red and green intensity than GM1SV3 baseline. Enzyme assays were performed and concluded that hydrolase activity is increased, and could be due to the autophagosomal changes in the cell.

These results confirm that there is the increased presence of lysosomes and autophagosomes along with lysosomal enzyme upregulation in both GM1SV3 cells and NSV3 starved cells. This connects lysosomal enzyme upregulation to impaired autophagy in neurodegeneration in GM1 Gangliosidosis and has the potential to be applied to other neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's. This discovery will open doors to tracking the progression of these diseases and allow individuals to be given the best treatment at the appropriate time.