Effects of lactate dehydrogenase in Alzheimer's Disease Using *Drosophila* Model

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Background

Alzheimer's disease is the most common neurodegenerative disease found in humans. The most prominent symptoms include memory loss and confusion, although there are several other cognitive, behavioral, phycological, and whole-body effects. Most people with this disease start experiencing symptoms around the age of 60 and symptoms progressively get worse as time goes on. It is thought that the cause of Alzheimer's is due to the aggregation of neurotoxic Aβ and TAU proteins. The buildup of these proteins is what leads to neurodegeneration and Alzheimer's symptoms.

Human Alzheimer's genes were implemented into fruit flies (*Drosophila melanogaster*) to examine the neurodegenerative properties of the disease since humans and fruit flies share a similar genetic makeup. The production of amyloid β in fly neurons was driven by using neuron specific promoter (Elav), and that was crossed with another line that carried human Aβ42. In previous experiments, it was found that down regulating certain genes allowed the transgenic flies to live longer and reduced their locomotor deficiencies (1). Microarray data showed differentially expressed genes and the genes of phenotypically rescued flies were selected for further testing. Lactate dehydrogenase (LDH) is one of the candidate genes that could be downregulated by utilizing LDH RNAi to rescue transgenic flies. LDH could also be downregulated by LDH inhibitor which is currently an ongoing research project.

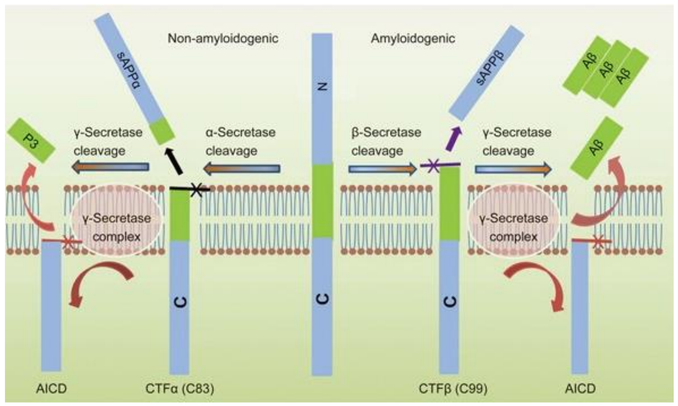

Figure1. Diagram of Amyloid Beta Production. Amyloid precursor protein (APP) is cleaved by one of two enzymes, α-Secretase or β-Secretase. Normally, APP is cleaved by α-Secretase, which cleaves it in the middle, followed by γ-secretase. This allows the APP to be soluble. Amyloid-β protein is generated when APP is cleaved by β-Secretase instead of α-Secretase. This cleaves APP in a different location which releases insoluble Aβ into the extracellular space (2,3).

Results

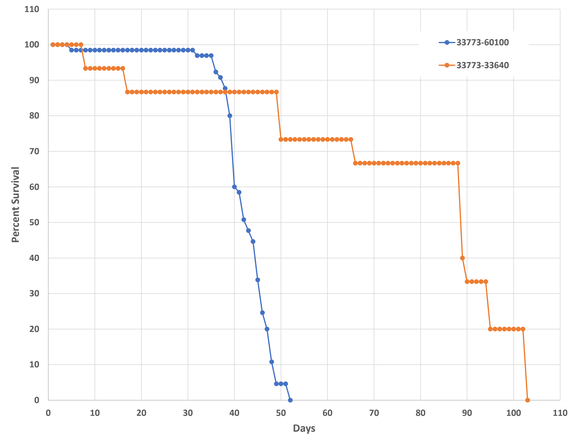

Figure 2. Lifespan of Alzheimer's (AD) flies in the presence of LDH RNAi (elav/33773/33640), compared to that of control (elav/33773/60100). Median lifespan for the former was 89 days, whereas in AD flies was 41 days.

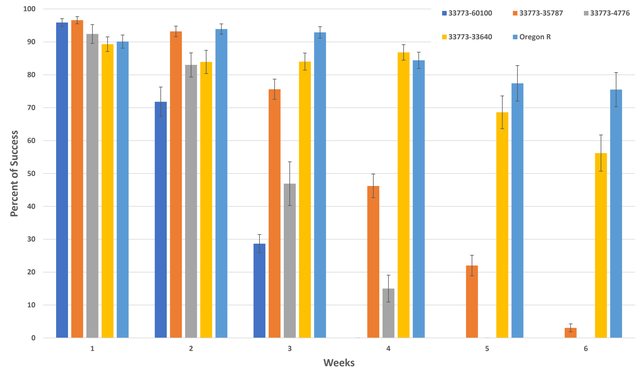

Figure 3. Locomotor function assessed by climbing assay. Knockdown of LDH (yellow; elav/33773/33640) rescued locomotor function, compared to three AD controls, either with vector insertion (elav/33773/60100), with UAS-mCherry (elav/33773/35787), or with UAS-GFP (elav/33773/4776). Oregon Red was included as wild-type control. Data is shown as mean with standard error (SEM).

Acknowledgement

The authors thank Dean Leverette and former Dean Roberts of Gupta College of Science, Dr. Hutchens of Biology, Honors program and Dr. Young of Grants and Research at Coastal Carolina University, South Carolina INBRE and NIH (5P20GM103499) for their generous support. Our lab is part of new Disease Modeling Research Center (DMRC) at Gupta College of Science of Coastal Carolina University.

Discussion and Future Direction

Lactate dehydrogenase (LDH) is an enzyme found in almost all body tissues that converts lactate to pyruvate and back. In the mammalian system there are two types of LDH. LDHA generates lactate in the astrocyte to transport to a nearby neuron. Then LDHB converts lactate back to pyruvate to use as an energy source (5). An increase in LDH gene expression as well as an increase enzymatic activity were observed in human AD patients as well as the transgenic flies. It was observed that an overexpression of LDH shortened the life span of flies while decreasing the expression of LDH increased life span (6). Our results show that knocking down RNAi through LDH inhibitors does successfully reduce the toxicity of amyloid Beta proteins. Both lifespan and locomotor function were rescued in the flies that had LDH knockdown. Contradictory results were acquired by Niccoli's group which found that LDH knockdown by RNAi did not rescue the locomotor motor function (7).

Our next step will be using LDH inhibitor, FX11, to mimic the effect of LDH RNAi in AD flies, and to quantify amyloid protein aggregates through immunohistochemistry and Western Blot analysis. Cancer cells are known to use LDHA to convert it to pyruvate. By knocking down LDHA with FX11, the progression of human pancreatic cancer and lymphoma can be greatly reduced (4). We hope by knocking down LDH in transgenic flies will rescue their altered phenotype and prevent Aβ from aggregating further.

Materials and Methods

Fly Stock

Transgenic Drosophila melanogaster lines were purchased from Bloomington Drosophila Stock Center (Bloomington, IN): elav-Gal4 (BL#458), UAS-Abeta42arc mutant (BL#33773), UAS-LDH RNAi (BL#33640), UAS-mCherry control (BL#35787), and UAS-nGFP control (BL#4776). Additional UAS-RNAi control lines (#60100) were purchased from Vienna Drosophila Resource Center (Vienna, Austria). All lines were kept in bottles and vials containing Jazz Mix food purchased from Thermo Fisher Scientific (Waltham, MA). A stabilized Amyloid Beta fly line was generated by crossing elav-Gal4 and UAS-Abeta42arc mutant. Virgin females from this line were collected and then crossed with males containing various RNAi lines as well as their controls. The F1 generations were collected and studied further.

Lifespan Analysis

Five vials with groups of 10 flies per vial was observed and recorded every other day. Flies were transferred to new vials every 5 days.

Negative Geotaxis (Climbing Assay)

Newly emerged male flies were housed in the cluster of five per vial, with total of 50 flies per experimental group. Climbing assays were conducted weekly. Flies are tapped to the bottom of a 15mL conical tube and timed for ten seconds. The percentage of flies that successfully reached the 8cm mark in ten seconds was recorded.

References

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