Mitochondrial Dysfunction in TDP-43 Knockout HeLa Cells

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Alzheimer’s disease (AD), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD) are all classified as neurodegenerative disorders. In many neurodegenerative diseases, TDP-43 leaves the nucleus and can be found aggregating in the cytoplasm of a cell. Mitochondria were selected as a target in this experiment because depolarization of the mitochondria's membrane potential can lead to apoptosis, linking this organelle to many neurodegenerative disorders. The first goal is to investigate whether mitochondrial clustering in TDP-43 knockout cells is due to the TDP-43 depletion from the nucleus. The second goal is to determine if mitochondrial clustering leads to mitochondrial dysfunction in TDP-43 knockout cells. The third goal is to identify mitochondrial genes that are differently expressed in HeLa knock-out cells compared to wild-type cells. When comparing the WT and TDP-43 KO HeLa cells, we observed a clustering of the mitochondria as well as a fragmentation of the mitochondrial network, visualizing through microscopy. Our studies showed that TDP-43 was able to successfully rescue the splicing in the TDP-43 KO HeLa cells after transfection; however, mutants of TDP-43 were not able to rescue this splicing. This was shown by performing a western blot and staining with NUP188 and EGFP. Membrane potential of the KO cells was lower than that of the WT, this depolarization can trigger neuronal death. Flow cytometry showed the membrane depolarization through staining WT and TDP-43 KO HeLa cells with TMRM. Our results supported our hypothesis as many mitochondrial genes and characteristics were differently expressed with the absence of TDP-43 splicing.

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