Parkinson’s diseased proteins in relation to autophagy

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Abstract

A cellular process of clearing your cells of damaged organelles is called autophagy. This process has been linked to neurodegenerative diseases such as Parkinson's disease. A factor of Parkinson's disease is a buildup of damaged proteins in the cell, including the proteins alpha synuclein and LRRK2. Beclin1 is a protein that is thought to activate autophagy. The purpose of this study was to determine if the absence of Beclin1 would lead to a buildup of more diseased proteins, thus indicating that autophagy was inhibited. By performing a series of Western Blot experiments, it was found that the specific Parkinson's related proteins, alpha synulcein and LRRK2, had a significant decrease when Beclin1 was not present. This indicated that the role of Beclin1 significance goes beyond affecting levels of diseased proteins. Further studies will need to be conducted to determine the characteristic of Beclin1 that affects the specific levels of alpha synuclein and LRRK2.

Introduction

The word 'autophagy' is derived from 'phagy', which means eating, and 'auto', meaning self. This cellular 'self eating' process is better described as the metabolic breakdown of damaged proteins or organelles in eukaryotic cells (Klionsky, 2007). In the 1960's, Christian De Duve, who discovered the lysosome, was the first to describe this process and coined the name autophagy. Throughout the past decade, autophagy research has rapidly expanded and increasing numbers of

studies have linked the process to a wide range of physiological phenomenon such as neurodegenerative diseases, ageing effects and diabetes (Klionsky, 2010).

The three major types of autophagy are macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). CMA is limited to a subset of proteins, while micro and macroautophagy are involved in "dynamic membrane rearrangements resulting in the ability to enwrap larger structures, such as organelles" (Yen, 2008). Microautophagy involves the direct invagination of damaged proteins or organelles into lysosomes without autophagosome involvement. Macroautophagy, here after referred to as autophagy, is the main focus of this research paper. In autophagy, autophagosomes, or double-membrane cytosolic vesicles, impound the cytoplasm and carry the cargo to the lysosome for degradation (Yen 2008). Autophagy can be activated upon starvation conditions, and when induced, the phagophore forms. The phagophore then envelops damaged organelles, proteins, and surrounding cytoplasm, and forms the autophagosome. Lysosomes combine with the autophagosome to make an autolysosome, which then degrades the autophagic substrates. The protein Beclin1 is essential for autophagy. However, the role of Beclin1 in autophagy and its relationship to disease-related proteins is not well understood.

The link of autophagy to neurodegenerative diseases, such as Parkinson's disease has been verified by past studies (Cheung, 2009). Parkinson's disease is a neurodegenerative disorder of the central nervous system, appearing when certain nerve cells of the brain die or become impaired. Specifically, it results from the death of dopamine-containing cells in the substantia nigra, a region of the midbrain where the cause of cell death is unknown (Cheung, 2009). Neurodegenerative diseases, such

as Parkinson's disease, cause a toxic buildup of protein in the cells, which cause them to become unstable. Parkinson's disease causes a buildup of diseased proteins in neurons. Researchers at the National Institute of Health uncovered the role of alpha synuclein as being a Parkinson's disease-related protein, while an international research team of neuroscientists at Mayo Clinic in Florida, uncovered the role of LRRK2, a protein that is believed to be the cause of the most common inherited forms of Parkinson's disease (Yue, 2010).

Autophagy is thought to clear out damaged proteins and Beclin1 is involved with activating autophagy. The purpose of this study was to determine if the absence of Beclin1 would lead to a buildup of more diseased proteins. The proteins looked at during autophagy activation were LRRK2 and alpha synuclein because of the significant role they play in Parkinson's disease.

Methodology

A Western Blot was performed to look at changed protein levels. Beclin1 knockout (-/-) or wildtype control (+/+) mouse embryonic fibroblasts (MEF) were grown in a cell culture medium containing serum, amino acids, and protein. After removing the culture medium, the cells were washed with Phosphate Buffered Saline (PBS). The PBS was then removed. Either 600 microliters (μl) of lysis buffer was used for the control samples or 400 μl of lysis buffer was used for the Beclin1

knockout. The samples were incubated in lysis buffer in a cold room, on a rocker for 10 minutes. The protein concentrations were then measured and the samples were place into eppendorf tubes. Once the samples were placed in the tubes, buffer containing SDS was added and the samples were heated to 95 degrees Celsius.

Protein samples and protein marker ladders were loaded into a gel and separated by gel electrophoresis for one hour at 30 Volts (V). The proteins were transferred onto a membrane at 15 V overnight in a cold room. The next day, membranes were incubated in blocking buffer at room temperature. Different detection protocols were used for different proteins; since the protein sizes of LRRK2, Beclin1, and alpha-synuclein are different (250 kiloDalton (kDa), 60 kDa, and 15 kDa respectively), the membrane was cut into three pieces using the protein ladder as a guide.

For Beclin1 and alpha-synuclein detection, membranes were blocked in buffer containing 5% milk in PBS + 0.1% Tween-20 for four to six hours while shaking slowly at room temperature. Anti-mouse Beclin1 made in rabbit and anti-mouse alpha synuclein made in mouse were diluted in milk at a concentration of 1:1,000 and incubated overnight. The next day, membranes were washed in PBS for one hour, changing PBS every twelve minutes using 3000 μl. Following one hour incubation with either goat anti-rabbit or goat anti-mouse secondary antibodies, membranes went through second wash for 1 hour, changing 3000 μl of PBS every twelve minutes. Membranes were dried and placed into a cassette and exposed to film imaging in a

dark room. Films were exposed for one minute, thirty minutes, and overnight, respectfully.

A more sensitive detection protocol was used for LRRK2 since it is more difficult to detect. LRRK2 membranes were blocked in Odessey Blocking Buffer for one hour. Anti-mouse LRRK2 made in rabbit was diluted in blocking buffer + 0.1% Tween-20 at a concentration of 3:3000 overnight in a cold room. The next day, membranes were washed in PBS. Next, goat anti-rabbit fluorescent secondary antibody was put in Odessey Blocking Buffer with 0.1% Tween- 20 and 0.01% SDS, and was diluted at 1:15000 while shaking for one hour at room temperature. Following more washing, LRRK2 membranes, were scanned by the Odessey reader to detect fluorescent signals.

The same steps were taken in a second experiment to test LRRK2 and alpha synuclein again.

Results

In the first experiment, LRRK2 was cut around 75 kDa. However, since the protein ran over 75 kDa, no proteins could be detected. Alpha-synuclein was cut at 25 kDa and the proteins were detected around 15 kDa. As shown in figure 1, there was more protein expressed in the wildtype cells then in the Beclin1 knockout cells.

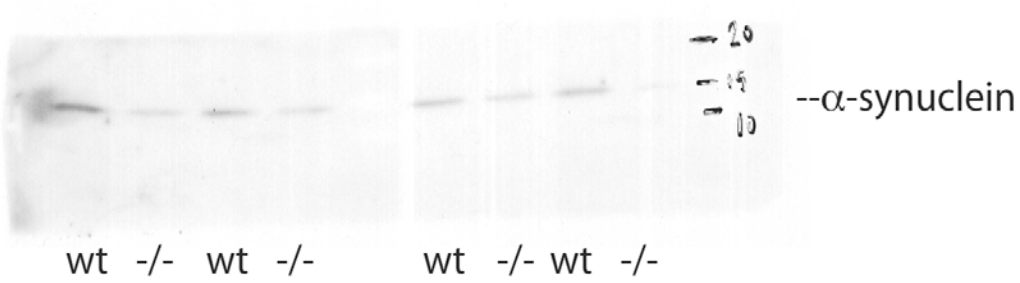
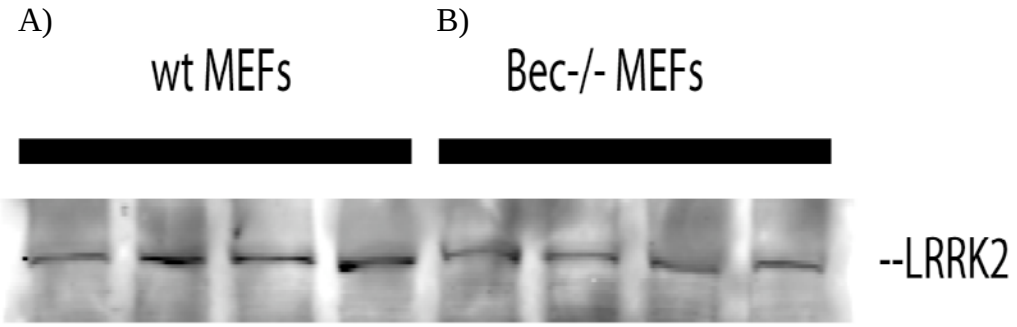


Figure 1: This image shows alpha synuclein protein expression in wt (wildtype) and -/- (Beclin1 knockout) cells. When the band is darker, there was more protein expression in the cells. This image shows that wt cells expressed more protein.

The second experiment tested for the presence of LRRK2 and alpha-synuclein in control wildtype and Beclin1 knockout cells. Figure 2 shows the Western Blot results for these two proteins. As shown, there was less LRRK2 and alpha-synuclein protein expression detected in the Beclin1 knockout then there was expressed in the control wildtype cells.



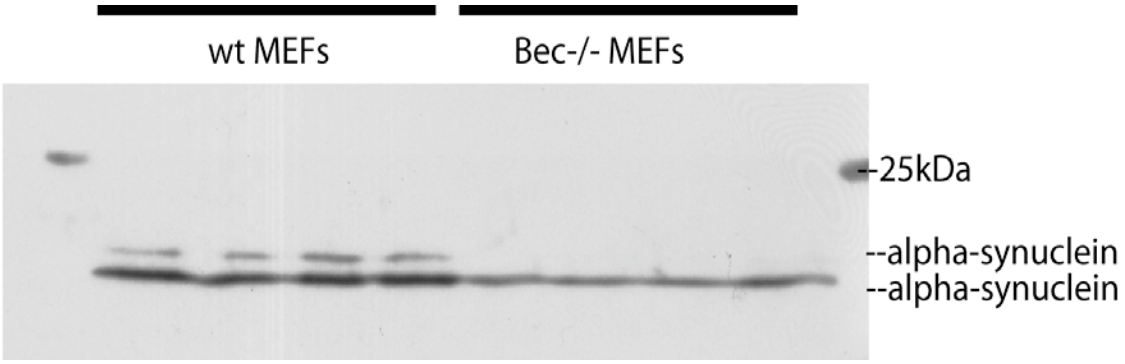


Figure 2: This image shows both LRRK2 (top) and alpha synuclein (bottom). There seems to be less expression of LRRK2 in the Beclin1 -/- (B) than in the wt (A). The two bands shown for the alpha synuclein (C) were originally thought to be a background band, but is actually showing less expression of alpha synuclein in Beclin1 -/- (D) cells.

Discussion/ Conclusion

The purpose of this experiment was to see if there were more or less diseased proteins in the Beclin1 knockout cells when compared to the control wildtype cells, which had no Beclin1 present. The absence of Beclin1 in the wildtype cells would suppress autophagy, and as we hypothesized to lead to more Parkinson's disease proteins accumulating in the cells. The proteins alpha synuclein and LRRK2 are a major factor of Parkinson's disease and were expected to be of higher concentration in the wildtype cells. Results for both trials showed higher concentrations of both LRRK2 and alpha synuclein in the cells containing Beclin1. This indicates that the role that Beclin1 plays in existence of these proteins goes beyond affecting the levels of alpha synuclein and LRRK2. Similar experiments should be conducted using neuronal cells to determine if the lack of Beclin1 has the same effect- since it is protein buildup in neuronal cells that leads to Parkinson's symptoms. This research is just a part of the "big picture". Further studies are needed to determine the

characteristic of Beclin1 that is affecting the levels of alpha synuclein and LRRK2.

Resources

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