Iron, α-synuclein and Autophagy in Parkinson’s Disease

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ABSTRACT

Iron is a vital element to nearly all kinds of organisms and iron overload has become one of the key underlying factor in the pathogenesis of neurodegenerative diseases. It was shown that iron together with excess cytoplasmic dopamine in neurons is associated with the aggregation of α-synuclein in cells. Although α-synuclein is a highly conserved protein among vertebrates, however, it has been found to aggregate in Lewy body as one of the hallmarks of Parkinson’s disease. We hypothesized that neurons transfected with mutant form of α-synuclein, after overloading with iron, will undergo excessive protein aggregation, resulting in cellular toxicity and hence triggering cell death via autophagy. Autophagy is the primary mechanism of breaking down entire organelles or large protein aggregates. Western blot and immunofluorescence methods were employed in this study to investigate autophagy after cells were overloaded with iron. We observed cellular morphological changes as well as abnormal aggregation of α-synuclein in iron treated cells as observed by immunofluorescent microscopy. Autophagic marker, LC3, was also identified via western blot indicating cells treated with iron underwent autophagic activities. With results supporting the hypothesis, we propose that the mechanistic understanding of autophagy under the above mentioned circumstances should be further investigated.

INTRODUCTION

Iron has become a key player in the pathogenesis of neurodegenerative diseases. The accumulation of iron has also observed to be associated with several neurodegenerative diseases including Parkinson’s disease (PD) and Alzheimer’s disease (L. Zecca et al. 2004). Studies have
shown that iron together with free radical generator such as dopamine in neurons is associated with the aggregation of α-synuclein in cells overexpressing the particular protein (Natalie et al. 2005). Cells have developed a few efficient protective systems from the toxicity of damaged organelles or protein aggregates. Autophagy in mammalian cells is one of the cytoprotective measures against various metabolic toxicity or damages of organelles (F. Scarlatti et al. 2008). This study attempts to show that three key players, iron overloading, excessive protein aggregation and autophagy are associated in the pathophysiology of PD. We hypothesized that neurons transfected with mutant form of α-synuclein, after overloading with iron, will experience excessive protein aggregation, causing cellular toxicity and hence triggering autophagy. We employed a dual approach strategy which are western blot and immunofluorescence in this study to investigate the role of iron in autophagic activities in neurons.

MATERIALS AND METHODS

SH-SY5Y cell lines were used in this study. Transfection to develop four steady cell lines which were SH-wild type, SH-vector, SH-A30P and SH-A53T were done for experiments. Cells were subjected to seven days of iron treatment. Cell density and Viability were taken daily by trypan blue assay. Cells were then collected as pellet and western blot was then conducted. Also, another batch of samples treated with iron was subjected to incubation of primary anti α-synuclein antibody and secondary antibody tagged with Cy3 for immunofluorescence. Transient Transfection of LC3-GFP followed by observation under fluorescence microscope.

RESULTS

In cell viability assay, we noticed that the steady growth of cells ceased at around day 5 when cell density and viability were found to drop. We observed that although the general trend appeared to be reduction in cell viability after day 5, the cells were overall still considerably viable with above 50% of viability.

In immunofluorescence, it was apparent that morphological changes occurred among the treated samples upon iron treatment as compared to the untreated samples. However, fluorescent signals observed did not appear to be obviously different between untreated and iron-treated cells despite the obvious morphological changes.

In LC3-GFP transfection, sign of autophagy was exhibited by emission of punctate signal in the form of pronounced green fluorescence under fluorescence microscope.
In western blot, heavy form of activated Cathepsin D was detected in the iron treated samples, with signal increasingly stronger from SH-SY5Y, SH-A30P to SH-A53T. However results only displayed relatively weak signals instead of 2 distinct bands for LC3.

RESULTS AND DISCUSSION

The reduction of viability of transgenic cells observed is thought to be parallel with discoveries that mutations in α-synuclein cause familial form of PD; further strengthening the fact that α-synuclein plays a significant role in pathophysiology of PD (Natalie et al. 2000). The missense mutations of α-synuclein, A53T and A30P were discovered in early onset familial form of PD (Michel 1999). Soon after the discovery of A53T mutant in the rare familial form of PD, α-synuclein was then identified as the major components in filamentous and granular form in Lewy body of PD (Michel 1999; Natalie et al. 2000).

LC3, abbreviation of microtubule-associated protein light chain 3 is used mainly as the protein marker of autophagy in this study. LC3 is a mammalian homologue of Apg8p, autophagic protein found in yeast (Yasuo 2001). Cathepsin D, a lysosomal aspartate proteinase, has been shown to be associated with autophagy (K.E. Larsen et al. 2002). Results from studies mentioned above thus suggested the idea that LC3-II is important in the formation of autophagosomes and is degraded rapidly by lysosomal enzymes afterwards (I. Tanida et al. 2005). Due to the dynamism of autophagy in the cellular system, particularly the rapid conversion and degradation of LC3-II, we found it difficult to produce evident results showing autophagic activities utilizing western blot in this study.

It is understood that autophagy plays a huge role in cytoprotective mechanisms not only in cells with damaged organelles or aggregates of proteins, but also in cells experiencing various forms of stress (F. Scarlatti et al. 2008). Despite the powerful capacity of autophagy in sustaining cell survival, it was observed that under certain circumstances, cells undergo a death mechanism which does not resemble any hallmarks of apoptosis or necrosis but solely autophagy (F. Scarlatti et al. 2008). The question to ask here is if autophagy triggered by iron and α-synuclein functions to propagate a totally opposing effect of sustaining survival.

CONCLUSION

It has become clear to us that iron accumulation introduces toxicity to neurons via stimulation of abnormal protein aggregation. Therefore, the mechanistic understanding of the role
of autophagy to be either pro-survival and/or pro-death will be of interest for future investigations in order to further relate to the pathogenesis of PD.

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REFERENCES


