Research Review

AUTOPHAGY AS A NEURONAL HOUSEKEEPER – A REVIEW

Mohannad A. Elkhider*, Bob Chaudhuri

De Montfort University, The Gateway, Leicester, United Kingdom.

ABSTRACT

Correspondence:

Mohannad A. Elkhider, De Montfort
University; The Gateway,
Leicester, **LE1** 9BH United Kingdom

Plan: *The present review envisages the role of autophagy in supporting neuronal cell growth, development and remodeling. Neurons are more prone to protein aggregation due to their nature as exist in the cell cycle and in post-mitotic cells.*

Preface: *Autophagy is a cellular degradative pathway where unwanted and weary cytosolic components are recycled. Targeted elements are delivered to the lysosome for degradation. There are three different modes of autophagy named micro autophagy, macro autophagy, and chaperone mediated autophagy which are responsible for selecting and delivering cargo to the lysosome. The aggregation of certain proteins yields cellular toxicity which eventually leads to cell death and neurodegeneration. Therefore, the autophagic duty of continuously monitoring and clearing out aggregated proteins is indispensable in neuronal cells.*

Outcome: *The accumulation of autophagosomes is an established hallmark in a number of neurodegenerative diseases. However, this observation has triggered controversy where one opinion considers the activated autophagic pathway to act as an executioner by initiating neuronal cell death while the other explains the presence of autophagosomes as a final attempt by the cell to sustain viability against the increasing amount of stress.*

1. INTRODUCTION

Principally in an autophagic process the unwanted cellular components are delivered to the lysosome and are recycled to basic building blocks for the synthesis of essential proteins. With regards to neurodegeneration, autophagy plays a cytoprotective role against neuronal cell death when under pathological and physiological stress. Cells which are lacking basal autophagy, unable to keep up with the mounting stress which leads to neurodegenerative conditions characterised with extensive cell death. Moreover, autophagy is hired for its housekeeping duties of clearing toxic aggregation-prone proteins which are accountable for neuropathogenesis once they exceed a certain threshold. Paradoxically, over activation of autophagy could result in extensive self-digestion of essential cellular components which are vital for survival.

Thus, the future approach must provide means of maintaining a balanced autophagic activity within basal levels which is sufficient enough to serve the desired housekeeping duties without disturbing the function of vital cellular components. This would be of benefit especially to older patients since their cellular activities decline, including autophagy, as they grow older.

The abundant presence of autophagosomes in neuronal cells of patients suffering from neurodegenerative diseases is an established observation in many recorded cases. This phenomenon has triggered controversial opinions of whether the observed increase in autophagosomes serve as means of neuronal protection or can be regarded as a sign of an alternative form of cell death that diseased cells undergo. Moreover, it still remains unclear whether these autophagosomes exhibit autophagic overactivity or a sign of a defect in one of the end stage steps of the pathway causing the accumulation of these autophagosomes.

The difficulty lying in exploring the root pathological elements in neurodegenerative diseases is the pro-death versus the pro-survival struggle that cells experience for a long period during the end stage of disease. These conflicting elements where some promote survival while others favour cell death make it difficult to distinguish signs of cell death from cellular compensation to maintain viability (Nixon 2006). However, the emerging notion with regard to autophagy and neurodegeneration is that autophagy is responsible for providing essential neuroprotective properties that promote survival rather than it being classified as a hired executioner of cells once triggered (Wong and Cuervo 2010).

The present review will discuss current opinions in terms of the role of autophagy in neuroprotection and the relationship between autophagy and neuropathogenesis in Alzheimer's and Parkinson's diseases.

2. Cytoprotective duties of autophagy to arrest neurodegeneration

Autophagy takes part in the developmental stage of neuronal cells. Loss of the autophagy genes *atg6* and *atg7* accounts for diminished motor function, altered behaviour, disturbed movement coordination and significant depletion of cells mostly in the hippocampus, cerebellar and cerebral cortices. Moreover, *atg7* knockout mice live up to a period of four weeks only (Komatsu et al 2006).

One of the hallmarks of neuropathology which is shared by a number of neurodegenerative disorders is the progressive accumulation of aberrant proteins in neuronal cells. Intracellular proteins differ in their integrity; some proteins are quite delicate and are more prone to aggregation as cells grow older.

For instance the aggregation of beta-amyloid (βA4) manifests in *Alzheimer's disease*, while the aggregation of Huntingtin protein is the causative agent behind the development of *Huntington's disease* (Murphy 2002). The toxic potential of these proteins depends on their intracellular abundance; hence, an imbalance in their turnover rate directly impacts the manifestation of neurological disorders (Rubinsztein 2006).

2.1. The significance of basal autophagy in neuronal homeostasis

The notion of basal autophagy taking place in neuronal cells is a topic of debate since autophagic vacuoles are hardly visualised by electron microscopy or fluorescent labelling in neurons.

Recently, investigations by Komatsu et al (2006) confirmed a constitutive autophagic activity that takes place in neuronal cell lines without the influence of an external stressor.

The experiment was done via knocking out the two essential autophagic genes *atg5* and *atg7* in mouse models; severe neuronal deterioration was recorded under normal physiological conditions confirming the presence of basal autophagic activity participating in neuronal homeostasis. However, poor visualisation of autophagosomes in neuronal cells is drawn back to the cells' high efficiency in clearing out formed autophagic vacuoles as any delay in their recycling process might compromise the cells' viability.

Besides the canonical activation of autophagy during starvation, homeostatic imbalance caused by the accumulation of proteins can initiate the degradative autophagic housekeeping machinery which successfully transports proteins towards the lysosome. It was previously believed that the duty of the lysosomal pathway is only limited to long-lived proteins; however, the study by Korolchuk et al (2009) established a connection between inhibition of the lysosomal pathway and the accumulation of short-lived proteins destined for proteasomal degradation. Inhibition of autophagy causes accumulation of the autophagy adaptor, Sqstm1/p62, which interferes with the elimination of ubiquitinated proteins by obstructing their transportation to the degradative proteases.

These findings disprove the previous notion of categorising the two degradative pathways independent of each other and confirm a secondary role of blocked autophagy in down regulating proteasomal degradation of proteins. Nonetheless, the two pathways work conjointly each having its own advantage when targeting proteins. Proteasomal degradation is more efficient in eliminating short-lived proteins; on the other hand autophagy targets larger aggregated proteins that do not pass through the narrow pores of the proteasomal barrel (Ravikumar et al 2008)**.**

2.2. Neuronal cell physiology requires autophagy

The vulnerability of neuronal cells is explained by two factors. The first is that the soma of a neuron could range from 4μm up to 100 μm; this large cytoplasmic area demands more regulatory efforts than that required in smaller cell forms.

The second reason is that unlike other cell types which continuously regenerate, neurons exit the cell cycle early and exist as post-mitotic cells. Hence, as neurons age the house keeping duties of autophagy decline all together with other cellular activities. This correlates with neurons having substantial amounts of regulatory genes and proteins taking part in autophagic and endocytic pathways (Nixon 2005)**.**

2.3. Resources of autophagy to provide neuroprotection

Autophagy promotes neuronal cell viability mainly by suppressing apoptosis. Cells with efficient autophagic machinery are capable of maintaining viability either by recycling their own components to generate energy or by degrading aberrant organelles burdening the cell. Purkinjie cells also depend on autophagy for membrane trafficking and synaptic transmission. Cells deficient in autophagy are susceptible to axonal dystrophy and cell death as they lack the neuroprotective role of autophagy.

When under excitotoxic stimuli caused by neurological disorders, autophagosomes are hired at the site of injury as mediators of neuronal remodeling. This mechanism serves as means of damage control adopted by neuronal cells when their viability is compromised (Yue et al 2008).

Boya et al (2005) suggested that, pharmacologic or genetic inhibition of autophagy is coupled with an incline in apoptotic cell death during periods of limited nutrition as cells are incapable of coping with their energy expenditure. A relevant example is demonstrated in lysosomal storage diseases where poor elimination of defective mitochondria and ubiquitinated proteins triggers apoptosis.

Defects in lysosomal hydrolases halt the trafficking of aggregated proteins and mitochondria to the lysosome causing their accumulation in autophagosomes. Damaged mitochondrion, specifically the ones with an impaired membrane potential, releases ROS and cytochrome c into the cytosol; these elements signal for cell death as they are bonafide triggers of apoptosis (Nixon2006).

3. Autophagy in Neurodegeneration

Autophagy activation is selective to aggregated proteins than the wild type forms of that same protein. For instance the presence of α-synuclein, which is associated with Parkinson's disease, within normal levels, is not significantly influenced by pharmacologic inhibition of autophagy, whereas the aggregated form, contributing to Lewy body formation, accumulates significantly as autophagy inhibition takes place (Ravikumar et al 2008)**.**

An interesting observation is the potential of sequestering the autophagy inhibitor mTOR within one of the cytoplasmic inclusions formed by aggregation-prone proteins observed in neurodegenerative disorders. This explains the heightened autophagic activity as the inhibitory signals of mTOR are ablated. These findings could explain the reason behind the increase of autophagosome formation and accumulation at the end stage of neuronal cell death in the context of neurodegeneration caused by proteins prone to aggregation.
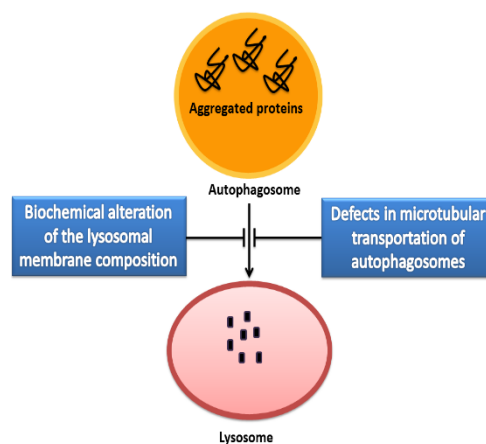
Pathways influencing autophagy: Microtubules

Fig.1: A.The first factor is the inefficient delivery of autophagosomes towards the lysosome due to defects in microtubular transportation. B. The second factor is the alteration of biochemical composition of the lysosomal membrane, this causing the block in the fusion step. C. The final outcome is the accumulation of autophagosomes which are unable to deliver their cargo.

Mainly autophagosomes depend on microtubules for transportation, developing molecular defects jamming the movement of autophagosomes throughout the cell was identified as the causative agent of spinal muscular atrophy and spastic paralgia. In this case, the efficiency of axonal transmission is compromised causing the localisation of autophagosomes in one region and being unable to travel towards the lysosome (Fass et al 2006; Larsen and Holzbaur 2006).

Dysregulation of the lysosomal capacity in degrading delivered cargo manifests numerous neurological disorders grouped under the name of lysosomal storage diseases (LSD). One possible cause of poor lysosomal degradation of delivered cargo in LSDs is the alteration of the biochemical composition of the lysosomal membrane.

Increased levels of the two lipids, cholesterol and glycosphingolipids, also referred to as gangliosides, is an evident symbol in LSDs (Futerman and van Meer 2004). These two lipids are essential components of the special membranous bodies known as the lipid-rafts or bundles. Hence, peculiar accumulation of cholesterol and glycosphingolipids in LSD is directly linked with an increase of lipid-rafts. As these rafts increase in number, the dynamics of autophagosome fusion to the lysosome is altered. Eventually, autophagosomes accumulate in the cytosol as they are unable to fuse with the lysosome and deliver their sequestered cargo. (Sobo et al 2007).

3.1. Alzheimer's disease

Alzheimer's disease is the most prominent form of dementia characterized by substantial atrophy of brain regions including the frontal cortex, parietal lobe, cingulate gyrus and the temporal lobe. The main causative is substantial neuronal loss and synaptic transmission in the above highlighted regions of the brain. The pathogenesis of the disease is triggered by proteolytic cleavage of a transmembrane protein precursor under the name of amyloid precursor protein (APP) into smaller fragments. These smaller fragments are known as amyloid-β (Aβ or βA4) which accumulates in extracellular regions within the vicinity of neurons forming what is referred to as senile plaques. Although APP is essential for neuronal development and remodelling of traumatised cells, the Aβ produced from its cleavage is problematic as it is categorized as one of the major features of Alzheimer's disease (AD) (Priller et al 2006). Another significant observation in AD is the extensive accumulation of autophagosomes within degenerating swollen regions of dystrophic neurites. This is mainly due to defects in the developmental stages of autophagosomes as well as failure in making the lengthy journey towards the soma where most of the lysosomes reside (Nixon 2007). The molecular basis of damage caused by the accumulation of Aβ is linked with increased generation of ROS and lipid peroxidation leads to destruction of essential cellular organelles which eventually triggers cell death (Glabe 2001).

Basal autophagy in healthy cells constantly produces Aβ as an end product of the proteolysis of APP by these proteases; however, the formed Aβ is constantly degraded via successful fusion of autophagosomes, harbouring Aβ, with the lysosome. On the other hand, defects in the maturation and transportation of autophagosomes make it impossible for sequestered Aβ to end up in the lysosome which ultimately manifests AD, as shown in Figure 2. Furthermore, undelivered Aβ residing in the cytosol triggers a compensatory process where the cell over-synthesizes autophagosomes to overcome the accumulation of Aβ. However, this compensatory process only worsens the case since accumulating autophagosomes would serve as synthesis reservoirs that unceasingly generate pathogenic Aβ (Yu et al 2005).
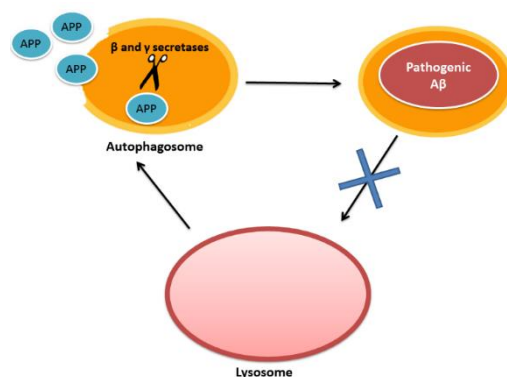
Fig.2

Intracellular levels of Beclin-1 (alternatively known as ATG6), which is required for autophagosome formation, are found to be consistently reduced in post-mortem AD brains. In a transgenic mouse model of AD that expresses human APP, genetic reduction of Beclin-1 expression stimulated Aβ accumulation and neurodegeneration due to a decrease in autophagy. Conversely, increased expression of Beclin-1 in APP transgenic mice significantly reduces amyloid pathology and neurodegeneration (Pickford et al 2008).

3.2. Parkinson's disease

Parkinson's disease (PD) is identified as the most prominent chronic disease compromising motor functions worldwide. Around 1% of the population above the age of 60 develops Parkinson's disease (Samii et al 2004). It is attributed to the selective depletion of neuronal cells storing dopamine in the mid-brain region known as the substantia nigra.

The pathogenesis of Parkinson's disease consists of unusual accumulation the alpha-synuclein forming insoluble protein aggregates known as Lewy bodies within neurons. In normal circumstances it is degraded via chaperone mediated autophagy. However, Mutants of α-synuclein, most importantly A53T and A30P block chaperones from binding wild type α-synuclein as they possess greater binding affinity to these chaperones than that of the wild type, therefore it is left to accumulate and form intracellular aggregates. Progressive accumulation of Lewy bodies is affiliated with an intertwined pattern of neuronal cell death involving apoptosis, necrosis, and the accumulation of structures resembling autophagosomes (Xilouri et al 2008). The available literature points out the two forms of autophagy, macroautophagy and chaperone-mediated autophagy (CMA), to be involved in PD.

3.3. Significance of autophagy as a therapeutic target in neurodegeneration

A striking finding by Komatsu et al (2006) confers the significance of basal autophagy in regulating stress in neuronal cells. Atg5 and Atg6 knockout mouse models were used to investigate the long term outcome of negating autophagy.

The tested mice showed hallmarks of neurodegeneration suggesting a house-keeping duty of autophagy that runs in the background to maintain homeostasis. Furthermore, the same investigators concluded that neuronal cell loss in a progressive manner is a consequence of inefficiency in the autophagic machinery coupled with cellular aging.

Maintaining basal autophagy by therapeutic means may be emerging as a useful tool to prevent neurodegeneration. As the whole pathway declines by age, a plausible precautionary intervention would be introducing more naturally occurring elements that up-regulate autophagy into one's diet (Pan and Ho 2008). Lycopene found in fruits and vegetables contain a red pigment, similar to the one seen in tomatoes and watermelon, is known to up-regulate autophagy.

Other natural products that could also be taken into consideration are: curcumin found in turmeric spice, resveratrol in grapes, lutein in green leafy vegetables, and epigallocatechin-3 in green tea.

3.4. Therapeutic targeting of autophagy in AD & PD

Pharmacologically enhancing autophagosomal synthesis in AD where defects in the maturation of autophagosomes and their movement toward the lysosome are the two main causatives behind the intracellular accumulation of autophagosomes. Inducing autophagosomes in this case would only exacerbate their accumulation and further increase the generation of the neurotoxic Aβ which is synthesized within autophagosomes.

In regards to PD, a feasible therapeutic approach would be through the over-activation of macroautophagy to target mutant α-synuclein. Only then would CMA be able to freely interact with the disease causative wild type α-synuclein and target it towards lysosomal degradation. The mutant forms of α-synuclein, A53T and A30P, inhibit α-synuclein elimination as they possess higher affinity to CMA. However, mutant α-synuclein variants are poorly degraded themselves by CMA and are better targeted by macroautophagy which is triggered as a compensatory response by the cell.

4. CONCLUSION

The up-regulation of the autophagic pathway might serve as a target for therapeutic intervention against neurological disorders arising from inefficient clearance of aggregate-prone substrates. The imminent investigational efforts must be dedicated towards the identification of molecular elements behind poor autophagosome maturation as well as agents which are responsible for facilitating the flux of autophagosomes in the direction of the lysosome. After identifying these elements a therapeutic strategy could be considered that would allow modulation of these factors in order to enhance the delivery of the sequestered cargo.

Over activation of autophagy could result in extensive self-digestion of essential cellular components which are vital for survival. Thus the future approach can provide means of maintaining a balanced autophagic activity within basal levels which is sufficient enough to serve the desired housekeeping duties without disturbing the function of vital cellular components. This would be of benefit especially to older patients since their cellular activities decline, including autophagy, as they grow older.

REFERENCES

1. Boya, P., González-Polo, R.A., Casares, N., Perfettini, J.L., Dessen, P., Larochette, N., Métivier, D., Meley, D., Souquere, S., Yoshimori, T., Pierron, G., Codogno, P. and Kroemer, G. Inhibition of macroautophagy triggers apoptosis. *Molecular and cellular biology.***2005**; 25 (3): p1025-1040.
2. Fass, E., Shvets, E., Degani, I., Hirschberg, K. and Elazar, Z. Microtubules support production of starvation-induced autophagosomes but not their targeting and fusion with lysosomes. *The Journal of biological chemistry.* **2006**; 281 (47): p36303-36316.
3. Futerman, A.H. and van Meer, G. The cell biology of lysosomal storage disorders. Nature reviews. *Molecular cell biology.* **2004**; 5(7): p554-565.
4. Glabe, C. Intracellular mechanisms of amyloid accumulation and pathogenesis in Alzheimer's disease. *Journal of molecular neuroscience.* **2001**; 17 (2): p137-145.
5. Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., Uchiyama, Y., Kominami, E. and Tanaka, K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature. 2006; 441 (7095): p880-884.
6. Korolchuk, V.I., Mansilla, A., Menzies, F.M. and Rubinsztein, D.C. Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. *Molecular cell.* **2009**; 33 (4), p517-527.
7. Larsen, E.C. and Holzbaur L.F. Axonal transport and neurodegenerative disease. *Biochimica et Biophysica.* **2006**; 1762 (11-12): p1094-1108.
8. Murphy, R.M. Peptide aggregation in neurodegenerative disease. *Biomedical Engineering.* **2002**; 4: p155-174.
9. Nixon, R.A. Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. *Neurobiology of Aging.* **2005**; 26 (3), p373-382.
10. Nixon, R.A. Autophagy in neurodegenerative disease: friend, foe or turncoat? *Trends in neurosciences.* **2006**; 29 (9): p528-535.
11. Nixon, R.A. Autophagy, amyloidogenesis and Alzheimer disease. *Journal of cell science.* **2007**; 120 (Pt 23): p4081-4091.
12. Pan, M.H. and Ho, C.T. Chemopreventive effects of natural dietary compounds on cancer development. *Chemical Society Reviews.* **2008**; 37 (11), p2558-2574.
13. Pickford, F., Masliah, E., Britschgi, M., Lucin, K., Narasimhan, R., Jaeger, P.A., Small, S., Spencer, B., Rockenstein, E., Levine, B. and Wyss-Coray, T. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *The Journal of clinical investigation.* **2008**; 118 (6), p: 2190-2199.
14. Priller, C., Bauer, T., Mitteregger, G., Krebs, B., Kretzschmar, H.A. and Herms. J. Synapse formation and function is modulated by the amyloid precursor protein. *The journal of Neuroscience.* **2006**; 26 (27), p7212-7221.
15. Ravikumar, B., Sarkar, S. and Rubinsztein, D.C. Clearance of mutant aggregate-prone proteins by autophagy. *Methods in molecular Biology.* **2008**; 445, p195-211.
16. Rubinsztein, D.C. The role of intracellular protein-degradation pathway in neurodegeneration. *Nature.* **2006**; 443, p780-786.
17. Sobo, K., Le Blanc, I., Luyet, P.P., Fivaz, M., Ferguson, C., Parton, R.G., Gruenberg, J. and van der Goot, F.G. Late endosomal cholesterol accumulation leads to impaired intra-endosomal trafficking. *PLoS one.* **2007**; 2 (9): e851.
18. Wong, E. and Cuervo, A.M. Autophagy gone awry in neurodegenerative diseases. *Natural neuroscience.***2010**; 13 (7), p805-811.
19. Yue, Z., Wang, Q.J. and Komatsu, M. Neuronal autophagy: going the distance to the axon. *Autophagy.* **2008**; 4 (1), p94-96.