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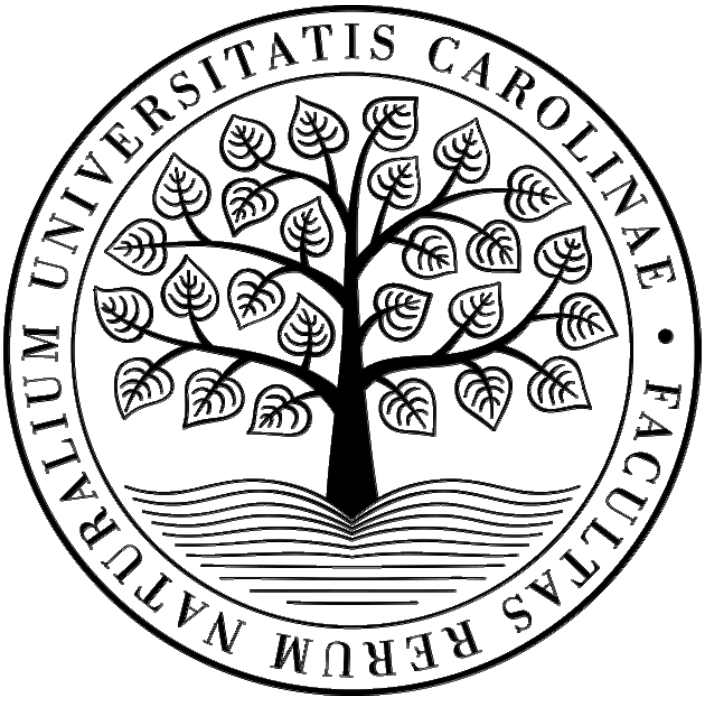

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The role of Autophagy in neurodegenerative diseases

Role autofagocytózy v neurodegenerativních onemocněních

Bachelor Thesis

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Prohlášení

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V Praze, 10. srpna 2018

Veronika Marková

Poděkování

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Abstract

Pro neurodegenerativní onemocnění, jako je Alzheimerova, Parkinsonova a Huntingtonova choroba, je charakterictická akumulace proteinů a nefunčních organel v cytoplasmě, které nejsou dostatečně odstraněny pomocí autofagocytózy. Ukazuje se, že bazální autofagocytóza, která za normálních podmínek zajišťuje homeostázu vnitřního prostředí, je narušena. Proces autofagocytózy není schopen odstranit všechny toxické částice při větším množství z důvodu úplné saturace tohoto systému a tak dojde k aktivaci jiných degradačních mechanismů, které mohou vést k obnovení homeostázy. Ovšem za některých okolností dochází k poškození neuronů, které vedou k jejích ztrátě. Úbytek neuronů v konkrétní oblasti mozku vede k pohybovým problémům i demenci. Lepší pochopení autofagocytózy a její role v patogenezi neurodegenerativních onemocnění by mohlo přispět k budoucí účinnější terapii těchto závažných chorob.

Klíčová slova: autofagocytóza, neurodegenerace, Alzheimerova choroba, Parkinsonova choroba, Huntingtonova choroba

Abstract

The characteristics of many neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington's disease is the accumulation of proteins and damaged organelles in the cytoplasm. Unfortunately, they are not sufficiently eliminated by autophagy. The basal autophagy, that maintains the cellular homeostasis, is disturbed in neurodegeneration. The process of autophagy becomes saturated and unable to remove all the toxic substances. Therefore, other degradation mechanisms are activated, aiming to restore the homeostasis. However, the neuronal cells are damaged under certain conditions leading to their death. The reduction in the number of neurons in specific brain areas may cause severe ataxias and dementias. Better understanding of autophagocytosis and its role v pathogenesis of neurodegenerative diseases may contribute to more effective treatment of these serious diseases in the future.

Key words: autophagy, neurodegeneration, Alzheimer's disease, Parkinson's disease, Huntington's disease

Contents

Abbreviations

Aβ	amyloid β peptide
AD	Alzheimer's disease
AMBRA1	Activating molecule in beclin-1 regulated autophagy
AMPK	AMP activated kinase
ATG	Autophagy-related gene
AV	Autophagic vacuole
BCL-2	B cell lymphoma
CMA	Chaperone-mediated autophagy
DN	Distrophic neurite
FIP200	FAK family-interacting protein of 200 kD
FLIP	FLICE-inhibitory proteins
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFP	Green fluorescent protein
GSPC	Glioma stem progenitor cell
HD	Huntington's disease
HSC70	Heat shock cognate 70 kDa protein
HTT	Huntingtin
IP3	Inositoltriphosphate
LAMP2A	Lysosome-associated membrane protein 2
LC3-I	Protein 1 light chain 3
LYNUS	Lysosome nutrient sensing machinery
3-MA	3- methyladenine
MEF2	Myocyte enhancer factor 2
mTORC1	Mammalian target of rapamycin complex 1
NSPC	Neural stem progenitor cell
NTD	Neural tube defect
PAS	Pre-autophagosomal structure
PD	Parkinson's disease
PI3K	Phosphatidylinositol 3-kinase
PI3K	Phosphatidylinositol 3-phosphate
PINK1	PTEN-induced putative kinase 1
PMP22	Peripheral myelin protein 22
ULK1	Unc-51 like autophagy activating kinase 1
UVRAG	UV radiation resistance-associated gene

1. Introduction

Neurodegenerative diseases like Alzheimer's, Parkinson's or Huntington's disease are currently prevalent in the aging population. The characteristics of neurodegeneration include neuronal damage and neuronal loss in specific brain regions. Neurodegeneration has a dramatic effect on the overall wellbeing of an organism and may result in altered mental functioning and ataxias. At the moment, neurodegenerative diseases are not curable, but we can establish some therapies slowing down the degeneration.

Autophagy is one of the degradation pathways that can be used to treat neurodegenerative diseases. There are many types of autophagy, but they all interact to maintain cellular homeostasis. The most important process is the basal autophagy (Hara et al., 2006). However, the basal autophagy is impaired in neurodegeneration. Even though the understanding of molecular mechanisms regarding autophagy is becoming more detailed every year, the pathological disturbances still remain a mistery.

One of the side effects of impaired autophagy is the accumulation of proteins and damaged organelles that are the major cause of toxicity for the neuronal cells. There are dystrophic neurites around the senile plaques composed of β-amyloid (Aβ) peptide and tau protein in Alzheimer's disease (Yu et al., 2005). In Parkinson's disease, Lewy bodies made up of α-synuclein and ubiquitin are observed in neuronal cells in many brain regions (Fujiwara et al., 2002). Mutant huntingtin aggregates are found in the neuronal tissue of HD patients (DiFiglia et al., 1997).

The role of autophagy in these neurodegenerative diseases will be discussed in detail including the molecular mechanisms. Indeed, some regulation mechanisms will also be described since they might be the site for potential treatment of neurodegenerative diseases.

2. Definition of autophagy

The term “autophagy” derives from the Greek word “self-eating” used by Christian de Duve in 1963, who also named the vacuoles to be “autophagic”. The term “autophagy” was used for the first time at a Symposium in London the same year (Klionsky, 2008). Some types of autophagy can be found in eukaryotes for example in yeast, plants and animals. The autophagy machinery is probably evolutionary conserved proving a useful mechanism for the cell to survive (Wesselborg & Björn, 2015).

Autophagy is one of the degradation pathways in the cell that is essential for the maintenance of homeostasis, cell differentiation and development and mainly its survival. It is believed that autophagy is more specific than other degradation pathways and has an adaptive function to protect the cell from neurodegeneration, aging other other pathologies (Levine & Kroemer, 2008).

There are major steps in autophagy process consisting of nucleation, elongation, maturation and degradation. There is a double membrane being formed around the vesicle and the autophagosome is delivered to lysosome for fusion to form autolysosome. The abnormal macromolecules including proteins, lipids and nucleotides are degraded using the autophagy-lysosomal pathway. This degradative pathway can also be applied to damaged organelles such as mitochondria and peroxisomes (Ghavami et al., 2014).

2.1 Specific autophagy

During the non-selective autophagy, the inner membrane of autolysosome is degraded together with the cargo and molecular building blocks are transported by lysosomal permeases back to the cytoplasm. On the other hand, autophagy might be very specific when pathogens or organelles need to be removed. The selective pathways of degrading different molecules include the xenophagy (intracellular pathogens), aggrephagy (aggregates of proteins), lipophagy (lipids) or zymophagy (secretory granules). The autophagic degradation of organelles include the ribophagy (ribosomes), mitophagy (mitochondria), ER-phagy (endoplasmic reticulum) and others. The autophagy might be induced under stress conditions like infection by pathogens, hypoxia, lack of nutrients or removal of growth factors needed for the development and maintenance of homeostasis (Wesselborg & Björn, 2015).

3. The types of autophagy

We can distinguish three subtypes of this process occuring in higher eukaryotes - the macroautophagy, microautophagy and the chaperone-mediated autophagy. These degradative pathways are in balance with the synthesis of the components including proteins to ensure the proper function of the cells (Mizushima et al., 2008).

3.1 Macroautophagy

Preferably, I'm going to talk about the macroautophagy for which we use the term "autophagy". The origins of this term are Greek where "auto" means "self" and "phagy" means "to eat" (Yorimitsu & Klionsky, 2005). This process in not selective compared to other degradational processes in the cell. This type of autophagy is the most conserved and can be found across organisms from yeast to humans. Macroautophagy is activated by protein aggregates, damaged organelles and cellular stress (Baba et al., 1997).

First, a part of a membrane becomes isolated in the cell that is known by the term phagophore or an isolation membrane. There is a preautosomal structure (PAS) created in yeast (Suzuki et al., 2001). This structure or the phagophore expands around the cytoplasm containing the structure for degradation (aggregated proteins or organelles) and encloses itself to form autophagic vacuole (AV) or an autophagosome.

Autophagosomes with the aggregated proteins in the cell are up to 1 μm in diameter. At this point, the autophagosomes can become part of the endocytic pathway, the late endosomes or the multi-vesicular bodies. Eventually, they encounter lysosomes. They fuse together forming the autolysosomes and the cargo is slowly degraded by acidic hydrolases. The cargo is broken down to amino acids that are taken by the transporters back to the cytoplasm. These amino acids are used in biosynthetic pathways to build new proteins. The autophagy has a central role in many processes including the degradation of proteins and pathogens, tumor suppression, antigen presentation, etc. (Hosokawa et al., 2009).

3.2 Microautophagy

Microautophagy is a mechanism described in yeast and is more direct than the other types of autophagy. The proteins are directly taken inside the late endosome using invagination. This process might be considered analogical with the

multivesicular body formation but the similarities are not yet clear (Sahu et al., 2011).

The tubular invaginations were observed not only in living yeast cells but also in isolated vacuoles. The vesicles budding from the tubules create autophagic bodies that are degraded by the hydrolases. The reconstruction of in vitro microautophagy has been done using the purified yeast vacuoles. There is a suggestion that ATG proteins might be involved in the regulation of microautophagy as shown in the cytosolic extracts from the starved ATG mutants (Kunz et al., 2004).

3.3 Chaperone mediated autophagy

In the third type of autophagy, the chaperons are involved in the identification of substances and they deliver them to the translocation complex on the lysosomal membrane for degradation. The chaperone mediated autophagy is more selective than the first two because the process relies on the binding of the heat shock cognate 70 kDa protein (Hsc70) to the cytosolic substrate proteins to form a complex. This complex binds to membrane protein associated with the lysosomal membrane, usually the lysosome-associated membrane protein 2 (LAMP-2A). The substrate proteins are unfolded and taken into the lysosome for degradation.

The researchers have found that knocking down the LAMP-2A protein results in the reduction of chaperone-mediated autophagy as well as the decrease of GAPDH and aldolase in dendritic cells. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase are the soluble cytosolic proteins in dendritic cells. Indeed, there was observed a significant decrease of protein degradation on the lysosomes (Sahu et al., 2011).

The CMA is activated when the levels of two major regulators, Hsc70 and LAMP-2A, increase in the cells. The activation of CMA is induced by increased stress, for example the starvation, oxidative stress or coming to contact with toxic substances. The LAMP-2A protein forms dynamic multimeric complexes that gather during different steps of the CMA. The targeting sequence of five amino acids LysPheGluArgGln is essential for the delivery of cytosolic proteins into the lysosomes for degradation (Bandyopadhyay et al., 2008).

4. Autophagy genes in mammals

There are five functional groups of ATGs in mammals and every system regulates different steps during the autophagosome biogenesis and they have an influence on each other as well. The five groups include ULK1 kinase complex, the Vps34/PI3-kinase complex, ATG9 complex, ATG18 homologs and ubiquitin-like conjugation systems. The autophagy genes are activated in the early stages of autophagy probably operating to form the autophagosome (Wesselborg & Björn, 2015).

4.1 UKL1 kinase complex

In the first group of UKL1-ATG13-FIP200-ATG101 complex, some orthologs of ATG1 were found. These include several uncoordinated movement like kinase (ULK) for example ULK1, ULK2, ULK3, ULK4. The knockout of ULK1 blocked the autophagy response. The FIP200 is a tyrosine kinase in the cytoplasm that contains nuclear localisation signal and therefore can be also found in the nucleus. ATG13 is a substrate of ULK1 so that it binds to its CTD and ATG13 (Chan et al., 2009).

The FAK family-interacting protein of 200 kD (FIP200) was found to be interacting with ULK1 and ULK2 at the autophagic membrane. ATG13 and FIP200 enhance the kinase activity and stability of UKL1. The complete interaction is needed for the recruitment ULK1 to the phagophore thus inducing autophagy. The ATG101 has not got any ortholog in S. cerevisiae and it interacts with ULK1 thought the ATG13 (Hara et al., 2008).

4.2 The Vsp34/PI3 class III kinase complex

The second group of Vsp34/PI3 class III kinase complex can be found in yeast, but in mammals has a function in autophagy and also in sorting the vacuolar proteins. The complex I contains Vps15, Vps 30, Vps34 and ATG14 (as shown in Fig. 1) and it can regulate the process of autophagy. In mammals, the PtdIns3K complex is composed of Vps15, Vps34 and beclin 1 (Kihara et al., 2001).

Beclin 1 contains many domains including the C-domain, coiled-coil domain and the BH3 domain. Beclin 1 iniciates autophagy because it promotes the vesicle nucleation. It also has an autophagy-specific domain called BARA (Ranaghan et al., 2017). This protein forms a core part of the phosphatidylinositol-3-kinase type III complex (PI3K) and has a key role in localizing its production to a specific parts of the membrane. Therefore the complex is involved in the membrane trafficking. This complex together with the protein kinase VPS34 and VPS15 produces the

phosphatidylinositol-3-phosphate (P13P) in vesicle membranes important for traficcing as well (Kihara et al., 2001).

The P13 kinase-beclin1 complex can recruit ATG proteins and their interaction is crucial for activation or repression of autophagy. The P13 kinase-beclin1 complex can interact with another ATG14 and UVRAG (Ultra-Violet Radiation Ressistance Associated Gene) associated with AMBRA (Activating Molecule in Beclin1 Regulated Autophagy) and this interaction supports the activation of autophagy. AMBRA1 is the activating molecule in beclin1-dependent autophagy as its deficiency in mouse emembryos causes autophagy impairment (Fimia et al., 2007).

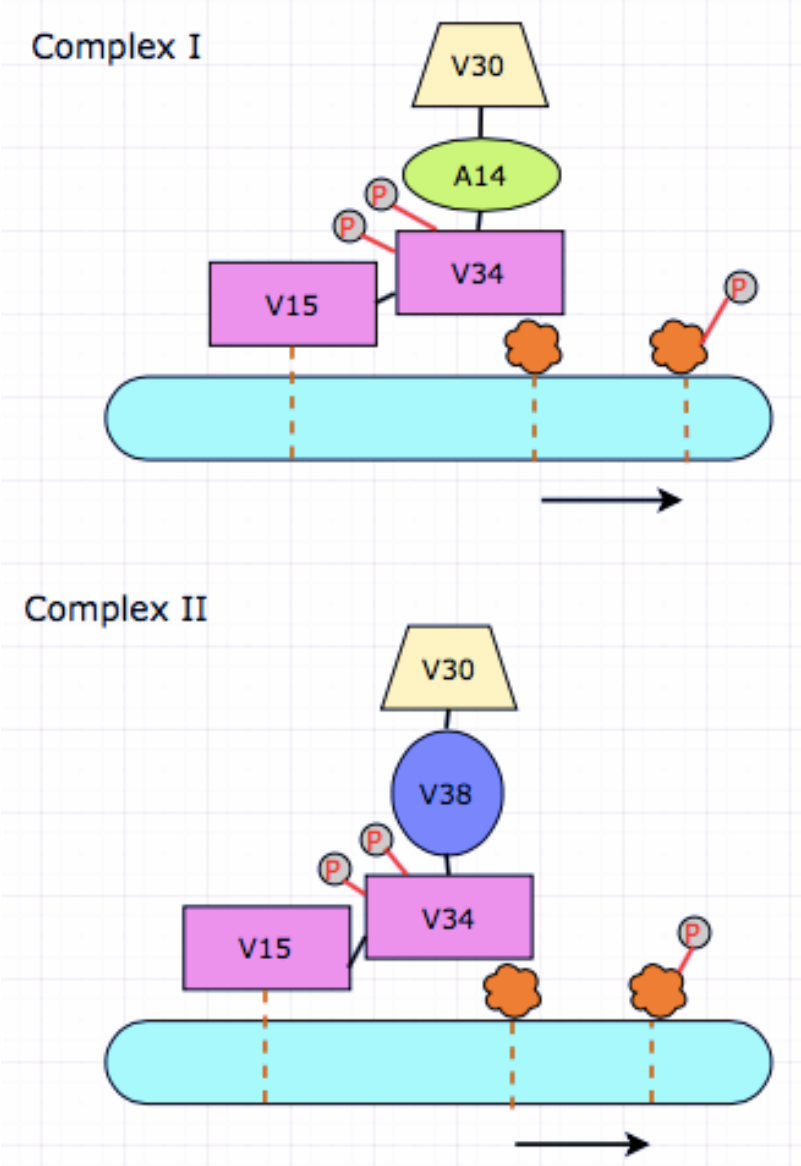

Figure 1
Model of PtdIns 3-kinase complexes
Complex I and Complex II are two different PtdIns 3-kinase complexes.
There are two different connectors A14 in complex I and V38 in complex II.
They mediate the interaction between two protein kinases - VPS30p and VPS34P. VPS15P phosphorylates VPS34P for their interactions as well as the interaction with the ATG14P/VPS38P.
Modified from (Kihara et al., 2001).

4.3 ATG9 recycling complex

The multi-spanning transmembrane protein ATG9 contributes to the de novo formation of the pre-autophagosomal structure called PAS. It has six transmembrane domains and is essential for the survival. There are ATG9 vesicles containing the ATG9 molecules formed in the cytoplasm. They eventually become part of the phagophore and the ATG9 clusters are recycled in the cytoplasm (Mari et al., 2010).

The ortholog of ATG9 called ATG9A was found in the trans-Golgi network. This ortholog changes its position when the cell is starving and integrates in the endosomal membranes that show positive autophagosomal marker GFP-LC3.

Other research argues that ATG9A is not integrated into the autophagosomal membrane but it only regulates the creation of phagophores. It is suggested that the localisation of ATG9A is regulated by WIPI2 and ULK1. However, some studies have demonstrated that ULK1 can localise the autophagosome formation independently on ATG9A during already mentioned parkin-mediated mitophagy (Itakura et al., 2012).

4.4 ATG18 homolog

The P13P protein has other effectors including the ATG18 proteins found in yeast. ATG18 plays a role in autophagy but ATG21 is rather involved in micronucleophagy. The both enable the intake of ATG8 to the site of autophagosome formation. ATG18 is in complex with ATG2 during autophagy but the autophagosome can be formed without the presence of ATG18. There are four homologs to ATG18 in mammals named WIPI1-4. WIPI1 and WIPI2 play a role in autophagy because they show the highest homology to ATG18. They allow a right localisation place to the phagophore and they also regulate the lipidation of LC3 (Proikas-Cezanne et al 2004).

4.5 Ubiquitin-like conjugation systems

Finally, there are two ubiquitin-like conjugation systems in autophagy. Both are involved in the expansion of autophagosomes and they show functional similarities. ATGs in both conjugation systems have ubiquitin-like functions and they are essential for autophagy. In the first system, ATG7 has the same function like E1 enzyme, ATG10 is like E2 enzyme and both are needed for an effective autophagy. ATG12 as the hydrophilic protein is activated by ATG7 and transferred to ATG10. ATG12 is then covalently attached to ATG5 and this complex associates with

ATG16 to form a huge multimeric complex as shown in Fig. 2 (Shintani et al., 1999).

The final multimeric complex ATG12-ATG5-ATG16 has a smaller size of approximately 350 kDa in yeast compared to more than two times bigger 800 kDa in mammals. The bigger size of this complex in mammals suggests that it might be composed of several sets of ATGs. Indeed, ATG16 is the major protein for ATG5 targeting and is probably localised in the pre-autophagosomal structure (Muzushima et al., 2003).

In the second ATG8/LC3 PE conjugation system, there is a prior step that preceding the activation of ATG8. ATG4 removes the C-terminal of ATG8 using its proteolytic activity. The second step becomes the activation of ATG8 by ATG7 and the complex is transferred to ATG3. ATG7 also serves the function of an E1 enzyme, but the E2-like enzyme becomes a different protein ATG3. ATG3 is conjugated with ATG8 through a thioester bond suggesting specificity of this interaction (Ichimura et al., 2000).

There comes the connection of the first and the second conjugation systems because the ATG12-ATG5 complex is the E3-like enzyme for ATG8. The lipidation of ATG8 is stimulated by ATG12-ATG5 thus activating the conjugation system (Hanada et al., 2007).

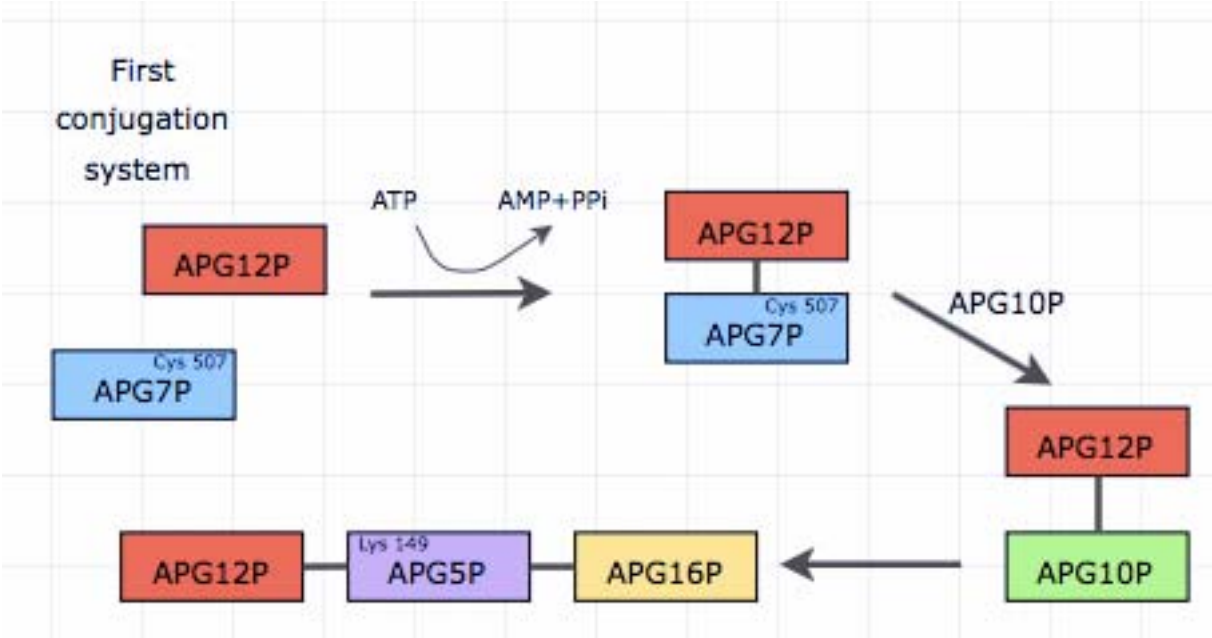

Figure 2 Model of APG12P-APG5P conjugation system

The formation of the multimeric APG12P-APG5P-APG16P complex in the first conjugation system. APG7P activates APG12P with the ATP hydrolysis and becomes attached at Cys 507 residue. The multimeric complex is formed after the interaction between APG12P and APG10P. Modified from (Shintani et al., 1999).

5. Regulation of autophagy

There are non-ATG proteins that influence the process of autophagy. These include the mammalian target of rapamycin (mTOR), Bcl2, beclin1, vacuolar sorting protein 34 (VPS34), AMPK and ULK1.

5.1 mTOR dependent induction

In yeast Saccharomyces cerevisiae, autophagy is activated directly by inhibition of TOR using rapamycin or amino acid starvation. There are two types of TOR (TOR1 and TOR2) and their inactivation has the same effect on cellular processes. When there is a lack of nutrients, the phosphatidylinositol kinase-related kinase TOR is activated. Rapamycin is an immunosuppressive drug that can also inhibit TOR thus inducing autophagy. As a result, there is an accumulation of autophagic vesicles (AVs) in both cases. Autophagy is inhibited in nutrient-rich conditions (Noda & Ohsumi , 1998).

5.1.1 mTOR1 in mammals

In mammals, the autophagic flux can be inhibited by many factors including high levels of insulin that reduce the concentration of amino acids. On the other hand, glucagon increases the amount of amino acids thus reducing autophagic flux. Autophagy is negatively regulated using kinase complex mTORC1. Under starvation conditions or the application of rapamycin, autophagy is induced by suppressing MTOR. The S6 protein is phosphorylated and decreased thus inducing autophagy (Blommaart et al., 1994).

The mammalian target of rapamycin (mTOR) restricts the activity of ULK which results in autophagy inhibition. First, it is necessary to form the pre-autosomal complex and this formation has not yet been described. The initiation complex is formed de novo from the ULK complex with at least four ATG genes ATG1, ATG9, ATG13, ATG17, multimeric complex and ATG6. Rapamycin intensifies the kinase activity of ATG1 and hyperphosphorylates ATG13. The hyperphosphorylation decreases the afinity of ATG13 to ATG1 thus repressing autophagy (Jung et al., 2009).

To initiate autophagy, the mTORC1 dissociates from the mega complex and both ULK1 and ATG13 become dephosphorylated. ULK1 autophosphorylates and is able to phosphorylate ATG13 and FIP200 thus inducing autophagy. This model tells us that the phosphorylation of ULK1, ATG13 and FIP200 depends on the action of mTOR (Jung 2009).

The phosphorylation of ATG13 mediated by ULK1 is necessary for the release of ATG13 and its recruitment to damaged mitochondria where it has a major contribution to parkin-mediated mitophagy (Joo et al., 2011).

5.2 mTOR independent induction

There are other ways to activate autophagy that are independent of mTOR. These include the beclin1, AMPK and FLIP proteins.

5.2.1 Beclin

The autophagy effector beclin 1 is one of the first identified autophagy proteins that has around 30% of identical sequences with ATG6. It is a 52 kDa protein that has a coiled-coil structure and is a key component of phosphatidylinositol 3-kinase (PI3Kc3). The core function of beclin1 is the nucleation of autophagosomes and regulation of membrane trafficking. The BH3 domain composed of 105-128 residues is the region involved in binding other molecules for example Bcl-2s. The beclin1-dependent autophagy is inhibited by murine γ-herpesvirus (γHV) Bcl-2s or its homolog M11 (Sinha et al., 2008).

Beclin 1 is an adaptor protein that can stimulate or suppress autophagy. When in interaction with Vps34, this complex is important for formation of autophagosomes. Furthermore, if the cells fail to produce beclin 1, the vesicles during autophagy would not accumulate due to failure to be properly localised. This impairment of autophagy caused by beclin1 deficiency was observed in Alzheimer's disease patients (Salmien et al., 2013).

5.2.2 AMPK and the role of Ca^{2+}

AMP-activated kinase (AMPK) is a positive regulator of autophagy that increases the autophagic flux when the levels of Ca^{2+} or IP3 decrease. The endoplasmic reticulum is the organelle that stores the major amount of intracellular Ca^{2+}**.** The IP3 receptors control the release of Ca^{2+} from the endoplasmic reticulum. B Hence, blockade of the IP3 receptors also inhibits the basal autophagy (Kania et al., 2017).

Glucose starvation triggers a response in the cell. AMPK activates ULK1 by its phosphorylation on Ser 317/Ser 777 inducing autophagy (as shown in Fig. 3). ULK1 is the autophagy-initiating kinase and the interaction between ULK1 and AMPK is promoted using rapamycin treatment (Kim et al., 2011).

5.2.3 FLIP proteins

There are two types of FLICE-inhibitory proteins; the viral type vFLIP and the mammalian cellular homologue cFLIP. Both of them can suppress rapamycin-induced autophagy as they bind to ATG3. There is a strong interaction with ATG3 when competing with LC3. LC3-I can be converted to LC3-II but cannot bind to ATG3 which prevents the autophagosome formation so the levels of detected LC3-II remain low (Lee et al., 2009).

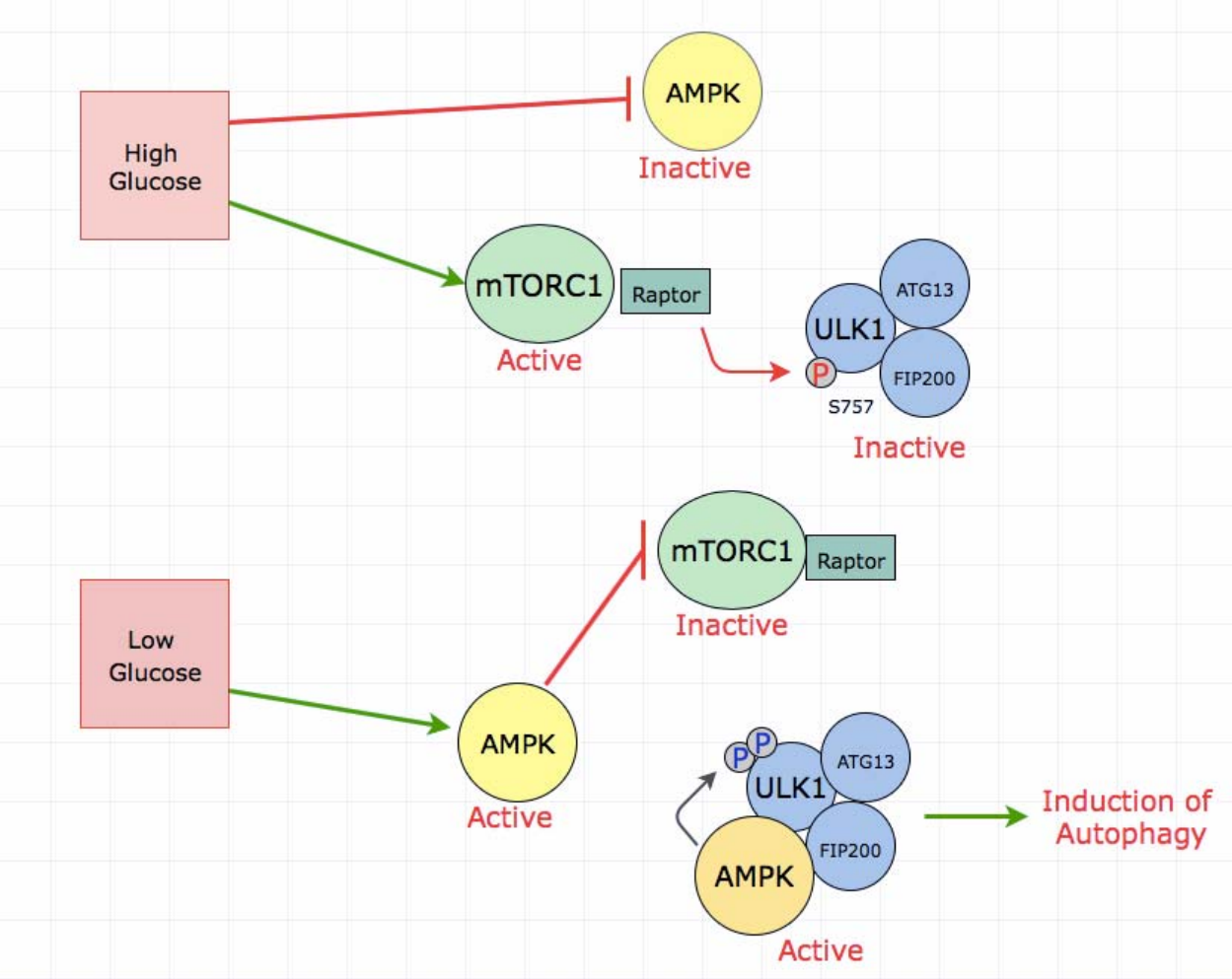

Figure 3

Model of ULK1 regulation by mTORC1 and AMPK

Upper panel: When there is high level of glucose in the neuronal cell and thus enough energy, mTORC1 is activated and phosphorylates tULK1 at serine 757. The phosphorylation prevents the interaction between AMPK and UKL1 complex.
Lower panel: When there is a low level of glucose, AMPK is activated and interacts with ULK1. mTORC1 is inhibited by phosphorylation of Raptor using AMPK.
The phosphorylated ULK1-AMPK complex induces the autophagy.
Modified from (Kim et al., 2011).

6. Autophagy in neuronal cells

In the case of neuronal cells, autophagy may be adapted to different compartments (the soma, the dendrites or the axons). The basal autophagy is essential for neuronal survival. The maintenance of the axonal homeostasis is the major function of basal autophagy in neuronal cells and neurons quickly degenerate when autophagy is impaired. Neurons are large polarised cells that are very sensitive to aggregates of any type (Hara et al., 2006).

6.1 The role in the development of the nervous system

Autophagy is an important process during neurogenesis as it is related to the differentiation of the cells in the nervous system. Neural tube is produced by the process of neurulation from the neural plate. The precursor cells are originated in the neural tube and a proper function of the brain is dependent on an accurate differentiation and migration of the cells to specific brain areas (Chenn & McConnell, 1995).

The deficiency of autophagy regulator AMBRA1 in the early stages of embryonic neurulation results in the development of neural tube defects (NTDs). The NTDs are caused by an incomplete closure of the neural tube during neurulation. The role of the regulators of autophagy such as protein kinase C (PKC) or AMP-activated protein kinase are still contradictory. There was a weakened autophagy in the rhombencephalon, prosencephalon mesencephalon in the exencephalic embryos observed as lower LC3-GFP levels (Wang et al., 2017).

There is a significant difference in the rate of autophagy between the neural stem/progenitor cells (NSPCs) isolated from the human fetal brain tissue and the glioma stem/progenitor cells (GSPCs). Autophagy is diminished in GSPCs compared to normal autophagy activity in NSPCs where autophagosomes are observed under TEM. Indeed, Western blot analysis confirmed higher LC3-II/LC3-I ratio in NSPCs than in GSPCs. However, when the GSPCs were stimulated for differentiation, autophagy was induced as LC3 positive vacuoles were detected in the cytoplasm (Zhao et al., 2010).

6.2 Autophagy and homeostasis

There is an evidence that autophagy has a central role for neuronal cells during their development and it is also crucial for maintaining cellular homeostasis. The autophagosomes and autolysosomes travel in anterograde and retrograde direction. The autophagosomes are created preferably at the distal axon and

undergoes the retrograde transport via dynein motors to the soma of the neurons, where the lysosomes are stored (Katsumata et al., 2010).

The organelles and a part of cytoplasm is degraded using autophagy, where the key step is the fusion of autophagosomes with lysosomes to form autolysosomes. The ATPase proton pump contributes to the change in the internal environment which becomes more acidic, thereby activating the acid hydrolases including cathepsins inside the lysosomes. The axonal transport of organelles using autophagy pathway is slowed down when the proteolysis of lysosomes is inhibited (Tanida et al., 2005).

The autophagosomes were detected in primary cortical neurons and the vesicles with the central protein for autophagy called LC3 were detected almost evenly distributed along the axons moving mostly in the retrograde direction. Indeed, the levels of LC3 were still kept low under normal conditions suggesting low levels of autophagy. It was suggested that the amount of autophagosomes is low, but there is a high rate of their turnover to maintain balance (Tanida et al., 2005).

6.3 Autophagy and neuronal death

The neuronal death can be induced by the loss of autophagy. This loss can occur in a specific region of the central nervous system or in specific types of neuronal cells. The protein aggregates or lack of nutrients promotes the survival of neurons by activating the autophagy. However, the degradation of large aggregates becomes difficult and the intracellular inclusions made up of aggregated proteins are observed in neurodegenerative diseases (Johnston et al., 1998).

The neural cells are very sensitive to protein aggregates and damaged organelles. They have to be removed by autophagy or any other programmed cell death to maintain active electrochemical signalling. Neurons also have a high energy consumption as they constantly transport proteins and organelles from the cell body to the axon ending and vice versa. There might be a failure in the induction of autophagy or the clearance of already formed autolysosomes causing their build up in the soma of the cells. Both disturbances play an important role in neurodegeneration. The dysregulation of autophagy may contribute to neuronal damage and possible therapy should be aimed at restoring the basal autophagy activity (Hara et al., 2006).

Autophagic stress is the imbalance between the formation of autophagic vesicles and their degradation. Long term state of not achieving the equilibrium means that the nonfunctional components of the cell are not eliminated are they build up in the cell causing cell death. The impaired lysosomal degradation also

leads to the imbalance. The large neurons such as motor neurons, Purkinje cells, sympathetic neurons and neurons in substantia nigra are specifically prone to autophagic stress (Chu 2006).

7. Autophagy in the neurodegenerative diseases

7.1 Autophagy in the pathogenesis of Alzheimer's disease

There are 30 million people worldwide living with the Alzheimer's disease and the scientists predict an increase of three times the number in around 20 years. The most common form of the disease is sporadic affecting the population over the age of 65. The disease is characterised by several changes including the accumulation of misfolded proteins, oxidative damage and neuroinflammation suggesting that autophagy might be an important process for regulation (Heneka et al., 2015).

A defining feature of Alzheimer's disease are neurofibrillary tangles and senile plaques composed of aggregated tau protein and the β-amyloid (Aβ) peptide. There is a plexus of dystrophic neurites (DNs) around and partly within the senile plaques (Yu et al., 2005). The dystrophic neurites are made up of swollen segments of dendrites and axons in the affected brains regions, for example in the posterior and anterior cingulate, hippocampus, thalamus and parietal cortex (Langbaum et al., 2010).

Using the immuno-EM analysis, the accumulation of autophagosomes was observed in the dystrophic neurites of neuronal cells suggesting that the macroautophagy is disturbed. The fact that the autophagosomes are rarely observed in healthy neurons with basal autophagy supports the argument that autophagy is induced in Alzheimer's disease. The macroautophagy is believed to have a neuroprotective function removing the toxins as well as the aggregated proteins to prevent the outbreak of neurodegenerative disease (Boland et al., 2008).

7.1.1 Macroautophagy in Drosophila melanogaster

The expression of the autophagy-related genes have been studied in Drosophila melanogaster as a model for Alzheimer's disease. The expression of these genes might be regulated by many factors including ageing or autophagy itself. First, it was suggested that the expression of genes regulating autophagy decrease during ageing. These genes include the ATG1, ATG8 and ATG18. The

expression of ATG18 was reduced in dfoxo and dsir2 mutants which means that they need to be active to sustain a required level of ATG18 (Omata et al., 2014).

To test the relationship between autophagy and Alzheimer's disease in a model of Drosophila melanogaster, the human Aβ42 was cloned and four mutants were created. Aβ42 promotes aggregates to develop in the cells contributing to neurodegeneration. Aβ42 was over-expressed in transgenic flies. Indeed, the expression of genes ATG1, ATG8 and ATG18 also declines with ageing. The results indicated that the expression of these genes were not affected by Aβ42. However, the neuronal toxicity as a result of Aβ42 over-expression was strongly enhanced by ATG1 showing the importance of this gene. There might be an auto-feedback mechanism of controlling the autophagy-related genes because ATG1 is able to decrease the ATG8 and AT18 and itself (Omata et al., 2014).

There is a close relationship between the lysosome activity and autophagy. Autophagy-lysosome pathway is believed to play a major role in neurodegeneration. The lysosome nutrient sensing machinery (LYNUS) monitors the level of nutrients in the cell. The mammalian target of rapamycin complex 1 (mTORC1) is part of the LYNUS machinery and phosphorylates transcription factor EB (TFEB) on the surface of the lysosome. The transcription factor EB does not reach the nucleus therefore the expression of autophagy-related genes as well as lysosome formation in inhibited. Indeed, the neuronal toxicity caused by increased levels of Aβ was enhanced when the expression of autophagy-related genes was reduced (Omata et al., 2014).

7.1.2 Macroautophagy in the mouse brain

Induction of macroautophagy contributes to neurodegeneration

Macroautophagy is induced in early stages of sporadic Alzheimer's disease before the accumulation of the Aβ peptide in transgenic mouse models. There was a marked accumulation of autophagic vacuoles (AV) in those mouse models with the human mutant PS1. The amount of autophagic vacuoles was 23 times higher in the neurons of PS1 transgenic mice compared to the controls. There were early and late autophagic vesicles found in the neurons but both did not mature to the later stages. The late stage autophagic vesicles contained a visible amount of multi-vesicular bodies indicating that they became the autophagolysosomes already. Indeed, the PS1 mice had twice the amount of LC3-II in the frontal cortex than the controls. LC3-II was present in the autophagolysosomes demonstrating the activation of autophagy (Yu et al., 2005).

Autophagy was reported to be activated in dying Purkinje cells in the cerebellum of lurcher mutant mice brains. These mice show degeneration in the cerebellar cortex mainly in the first four weeks of life. Electron microscopy was used to observe autophagosomes in the early and later stages of maturation. In fact, glutamate receptors are believed to be able to induce autophagy thus contributing to neurodegeneration (Yue et al., 2002).

Constitutive autophagy was observed to be active in cortical neurons taken from postnatal rat pups (Boland et al., 2008). Rapamycin was applied to neurons causing autophagy induction in 1 hour and elevation of LC3-II up to 90% in 24 hours. The total number of mature autolysosomes was much greater compared to controls. The placement of LC3 tagged by DsRed supports the observation that the fusion of autophagosome with lysosomes occurs right after its formation in the cell. The levels of LC3-II rose up to 444% after 24 hours and the number of autophagosomes increased compared to no rapamycin impairment. Therefore the induction of autophagy may be used as a therapeutic treatment for Alzheimer's disease because there is a massive build-up of autophagosomes. Induction of autophagy may improve the efficiency in maturation of AVs thus contributing to the clearance of toxic protein aggregates (Boland et al., 2008).

Suppression of autophagy contributes to neurodegeneration

There are studies demonstrating that the suppression of autophagy in neural cells might cause the development of neurodegenerative disease in mice. Autophagy is less selective in comparison to the ubiquitin-proteasome degradation system. The ability to survive under the starvation conditions is probably evolutionarily conserved. The deficiency of ATG5 and ATG7 genes in mice cause insufficiency of nutrients right after birth needed for normal energy metabolism in mice (Hara et al., 2006).

There is further evidence, that the impairment of autophagy might cause or at least contribute to the accumulation of abnormal nonfunctional proteins thus causing neurodegenerative disease in mice (Hara et al., 2006). Neural-cell-specific ATG5 mice were crossed with mice expressing Cre recombinase causing exon 3 to be deleted. This type of recombination was successful in 90% of neuronal cells in mice. The protein 1 light chain 3 (LC3-I) could not be converted to LC3-II due to the suppression of ATG5. There was no LC3-II detected in the cells because the autophagy was suppressed. Some neurons were lost in the cerebellum including the Purkinje cells and swelling was also observed. Swelling mainly in the axon area was distinct in many brain regions including the hippocampus, cerebral cortex, caudal

pons and both the anterior and posterior thalamic nucleus. Protein aggregation was also detected in the cytoplasm of neurons in the pons, medulla oblongata and thalamus (Hara et al., 2006).

These mutant mice evolved some behavioural and motor deficits before four weeks of age. The analysis of movement showed ataxic walking further supporting the motor deficit probably due to changes in the thalamus, pons and other brain regions. The behavioural deficit could be explained using the swelling in the hippocampus. The balance as well as the coordination of movement was also affected probably due to protein aggregates in the medulla oblongata and swelling in the cerebellum. These changes can be seen in mouse models with neurodegenerative disease (Hara et al., 2006).

7.1.3 The role of protein aggregates in neurodegeneration

Protein aggregates are associated with neurodegeneration. To test the removal of protein aggregates using autophagy, a point leucine to proline mutation was applied to the peripheral myelin protein 22 (PMP22) in the Schwann cells causing aggresomes to develop in the cells. The Trembler (TrJ) mice with this mutation developed aggresomes that could be cleared using autophagy. The autophagosomes full of aggregates were detected in the cytoplasm of the Schwann cells of TrJ mice. The late stage of autolysosomes could be visible in the same cytoplasm as well (Fortun et al., 2003).

However, not all Swann cells contained autophagosomes. The proteasome inhibitor lactacystin was applied to the Schwann cells of normal mice. This application resulted in aggresome formation but the cells recovered in normal growth medium after 24 hours. This testing suggest that aggresomes are transient structures that can be removed by autophagy under favourable conditions. The reduction in the number of cell due to cell death was not significantly different in normal SCs cells treated with lactacystin compared to the ones that had the washout period allowing regeneration (Fortun et al., 2003).

The removal of aggresomes using autophagy is not a process unique to Schwann cells as it was observed in fibroblasts. Indeed, autophagy is an ongoing process in TrJ mice (Fortun et al., 2003). Autophagy is the major process of the removal of aggresomes but there are probably other processes of degradation involved. After the inhibition of autophagy, up to 15% of the cells did not have any aggregates suggesting that there are other processes of degradation active in the cells. There was an evidence of decline in macroautophagy as well as chaperone-mediated autophagy with ageing of the animals. These pathways become less

efficient probably due to their saturation. As a result, there is an accumulation of aggregates even when autophagy is still partly active in the cells (Fortun et al., 2003).

The AVs in dystrophic neurites appear in large numbers in Alzheimer's disease patients. The AVs are surrounded by double-membrane and their appearance is multilamellar as well as dense compacted (Boland et al., 2008). The brains of Alzheimer's disease patients were compared to the data in this study. There is an obvious difference between the dystrophic neurites of primary cortical neurons where the autophagy was impaired compared to dystrophic neurites in Alzheimer's disease brains. However, the AV morphology becomes very similar when the autophagosome proteolysis is inhibited in primary cortical neurons or the fusion of autophagosome with lysosomes is impaired (Boland et al., 2008).

7.1.4 Macroautophagy in the human brain

The induction of macroautophagy specimens was studied in the human brains as well. As described above, we can detect the LC3-II to LC3-I ratio as LC3-II is present in the autophagosomes after the induction of autophagy. Using the Western Blot method, the levels of LC3-II were compared in grey matter of normal brains as well as in the brains in the early or later stage of Alzheimer's disease. Not surprisingly, as the Alzheimer's disease proceeds, the amount of LC3-II increases almost proportionally. The normal brains showed low levels of LC3-II suggesting a low level of autophagy (Yu et al., 2005).

The human brains were further studied in the laboratory conditions to investigate the role of autophagy in Alzheimer's disease. Specimens of the neocortex were studied using biopsy to compare brains of Alzheimer's patients and healthy control brains (Nixon et al., 2005). The specimens were studied from 7 patients using electron and immunoelectron microscopy after being incubated in primary antibody and subsequently with appropriate conjugated secondary antibody. Active lysosomal proteases were accumulated in neurites in brains with Alzheimer's disease. Many neurites had the entire intercellular environment filled with immature autophagic vesicles. The autophagosomes with double membrane did not have hydrolases compared to mature autophagic vesicles that already fused with the lysosomes. The autophagic vesicles of different stages of maturation were not only observed in dystrophic dendrites but also in the neurons with no pathological changes in brains with Alzheimer's disease (Nixon et al., 2005).

In general, the number of AVs observed was much higher than expected. These observations support the previous studies that autophagy is induced when

the brains show pathology of Alzheimer's disease. However, accumulation of AVs suggest that macroautophagy might be impaired later in some of the maturation stages due to blocking the transport of AVs to the cell body (Nixon et al., 2005).

When analyzing the control brains, the number of AVs was very limited suggesting low level of autophagy. The AVs were rarely visible in the axons or the dendrites. However, they were still present, because macroautophagy is an active process in neurons providing the regeneration of neurites. Properly functioning autophagy was observed in neuronal perikarya, but the number of autophagosomes was still much lower than its amount in Alzheimer's disease brains. The increase in the frequency of autophagosomes came up to twenty times higher the amount of AVs in the brains with Alzheimer's disease pathology. It is suggested that the autophagic-lysosomal pathology is more expanded and robust in Alzheimer's disease compared to any other neurodegenerative diseases (Nixon et al., 2005).

7.2 Autophagy in the pathogenesis in Parkinson's disease

Parkinson's disease is one of the most prevalent neurodegenerative diseases worldwide. There are two main features observed in the brains; the loss of neuronal cells and presence of Lewy bodies. Lewy bodies are intracellular neuronal inclusions that are composed of α-synuclein and ubiquitin (Fujiwara et al., 2002). Apart from loss of dopaminergic neurons (DA) in the substantia nigra (as a part of the basal ganglia) due to oxidative stress, Lewy bodies are observed in the thalamus, hypothalamus, cerebral cortex and brainstem in the PD brains (Zhang et al., 2000).

Neurotoxins like metamphetamine (MATH) or 3-methyladenine (3MA) producing the oxidative damage might cause the changes in the brains of Parkinson's disease patients. Dysfunctional autophagy might be responsible for the neurotoxicity or neurotoxicity might disturb the process of autophagy. Even though autophagy should protect the neuronal cells from building up the Lewy bodies, aberrant autophagy might cause neuronal degeneration (Larsen et al., 2002).

7.2.1 Autophagy in the mouse brain

The transcription factors called the myocyte enhancer factor 2 (MEF2A to MEF2D) have an influence on neuronal cell death and have been studied together with CMA in the relevance to neurodegenerative diseases like Parkinson's disease (Yang et al., 2009). The neurons are protected from death as observed in vitro in PD mouse models when MEF2 was enhanced (Smith et al., 2006). However, the degradation of MEF2D by CMA was attenuated when pathogenic and wild-type of α-synuclein were expressed. The degradation or inhibition of MEF2s caused neuronal

death suggesting that slowing down the CMA might be beneficial for the treatment of Parkinson's disease (Yang et al., 2009).

An impaired CMA in the culture of neuronal cells from mouse models showed higher sensitivity to cellular stress and potential development of Parkinson's disease (Massey et al., 2006). The expression of LAMP2A was blocked using using specific RNAi decreasing the CMA. Further reduction in LAMP2A degradation using 3MA neurotoxin inhibited macroautophagy. However, the rate of degradation of long-lived proteins was surprisingly higher than the levels in control control cells suggesting different degradation pathways activated (Massey et al., 2006).

There was an macroautophagy induction observed in transgenic mouse models with mutant A53T α-synuclein (Yu et al., 2009). The aggregated α-synuclein is efficiently cleared using macroautophagy suggesting a positive effect of this up-regulation. Indeed, the markers LC3-II and beclin1 significantly increased in the substantia nigra of SKO mice suggesting an increase of macroautophagy compared to the cortex where α-synuclein was not expressed (Yu et al., 2009).

7.2.2 Autophagy in the human brain

The substantia nigra pars compacta and the amygdala in PD and control brain samples have been studied to demonstrate the role of chaperone-mediated autophagy (Alvarez-Erviti L. et al., 2010). The knockdown of LAMP2A protein decreased CMA increasing the α-synuclein half-life. The levels of LC3-II increased suggesting induction of macroautophagy. This means that decrease of CMA with higher age raises the α-synuclein levels and cells are more dependent on macroautophagy to restore the balance in the internal environment.

A pentapeptide sequence 95VKKDQ99 was found to be consistent with recognition motif of CMA suggesting that α-synuclein might be degraded by the chaperone-mediated autophagy. The long-half life of α-synuclein was confirmed to activate both CMA and macroautophagy. Therefore the presence of CMA motif on α-synuclein does not guarantee its degradation using CMA. Mutations in the 95VKKDQ99 sequence reduced the protein association with the lysosomal membrane and its translocation to the lumen (Cuervo et al., 2004).

There is a difference in the wild-type α-synuclein that is degraded by CMA compared to the pathogenic α-synuclein that shows reduced CMA degradation even though it has a high affinity to CMA receptor (Cuervo et al., 2004). Disease causing α-synuclein has point mutations, for example Ala30Pro, Ala 53Thr or His50Gln, causing misfolding and aggregation (Xu et al., 2016). Both types of α-synuclein

increased the MEF2D levels in the brains of PD patients compared to controls (Yang et al., 2009). Slowing down the CMA degradation activates macroatophagy, but it is not enough to prevent formation of aggregates that may lead to neuronal loss (Cuervo et al., 2004).

To further support the argument that this induction of macroautophagy can be fatal to the neuronal cells, the human mutant A53T and WT ASYN were expressed in neuronal cells leading to CMA inhibition and macroautophagy induction that resulted in neuronal death. There was an increase in LC3-II, significant autophagosome formation and neuronal death reported probably due to the toxicity. However, the impaired CMA might still be major factor leading to loss of neurons (Xilouri et al., 2009).

The clearance of α-synuclein was promoted when macroatophagy was induced in human brain samples from Brodmann area 9 of the cerebral cortex (Yu et al., 2009). The LC3-II and beclin 1 markers were increased in patients diagnosed with dementia with Lewy bodies (DLB) compared to post-mortem controls of the same age. DLB is a different form of Parkinson's disease. The glia cells show very low levels of LC3-II suggesting that macroautophagy is minimal and predominantly induced in neurons (Yu et al., 2009).

7.2.3 Disregulation of mitophagy in PD

There are autosomal dominant and autosomal recessive genes (PARK2, PARK6, PARK7) linked to the development of familial variant of Parkinson's disease. These genes are linked to disfunction of mitochondria and the gene products like parkin and PINK1 are involved in mitophagy contributing to removal of damaged mitochondria (Lynch-Day et al., 2012).

Mitochondria are the sites of oxidative phosphorylation producing up to 90% of the energy in the cell. However, mitochondria are the major source of reactive oxygen species that can cause expressive damage when their production exceeds the available levels of protective antioxidants. The clearance of damaged mitochondria is necessary to prevent the neuronal death (Lynch-Day et al., 2012). One of the studies of postmortem brain tissues revealed that the activity of the complex I of the mitochondrial electron transport chain was reduced up to 50% in the neurons of somatosensory cortex and up to 27% in the prefrontal cortex in PD patients. Indeed, the amount of mtDNA was decreased in PD patients compared to controls probably due to mitochondria loss and impaired mitophagy (Gatt et al., 2016).

The reduced number of mitochondria in the neurons of the substantia nigra in PD patients have also been reported. Autophagy was found to decrease a concequence of silencing the expression of PINK1. Further over-expression of parkin increased autophagy or mitophagy, thereby contributing to the mitochondria removal (Gegg et al., 2014).

PINK1 has a neuroprotective role as it prevents the mitochondrial apoptosis when exposed to cellular stress. The mitochondria are maintained because of the serine-threonine kinase that has a mitochondrial import sequence on the N-terminal and interaction with Parkin. Silencing PINK1 raised the oxidative stress levels and activated the mitophagy (Michiorri et al., 2010). The full-length PINK1 (PINK1-FL) was over-expressed thus increasing LC3-II levels, which suggests activation of autophagy under starvation and even under nutrient-rich conditions in neuronal cells. On the other hand, parkin is able to enter directly the dysfunctional mitochondria activating mitophagy to eliminate them while silencing the PINK1 (Michiorri et al., 2010).

There is a strong interaction between PINK1 and beclin1 involving both N- and C- terminal regions. Both beclin1 and PINK1 are mainly observed in mitochondria but beclin1 is also detected in the ER and trans-Golgi network. To demonstrate the interaction between PINK1 and beclin1, 3-methyladenine blocked autophagy when applied to GFP-LC3 vacuole-positive cells. The knockdown of beclin1 also contributed to a reduction in autophagy in the cells where PINK1 was over-expressed. However, the amount of AVs was higher than that observed in control cells suggesting an activation of other types of autophagy (Michiorri et al., 2010).

7.3 Autophagy in the pathogenesis of Huntington's disease

Huntington's disease is a dominantly inherited developmental and neuromuscular disorder caused by expansion in the CAG repeats in the exon 1 on huntingtin gene (HD gene). This gene encodes a protein huntingtin but its function has not yet been fully described. The polyglutamine (polyQ) stretch as a product of CAG repeats becomes pathological to neuronal cells when exceeding the length of 37 glutamines. The aggregates of huntingtin protein have been observed in brains of HD patients (Möncke-Buchner et al., 2002).

There are major symptoms in Huntington's disease like cognitive loss, motor disturbance, anxiety and irritability. When the onset of the disease emerges during adolescence, the symptoms become more severe, leading to death in 10 to 20

years. The loss of neurons is visible mainly in the dorsal striatum, the caudate nucleus and the putamen (MacDonald et al., 1993).

The mutant huntingtin forms aggregates in the neuronal cells increasing the apoptotic processes and leading to toxic stress. Small fragments of huntingtin protein accumulate in the cytoplasm or passively diffuse into the nucleus if their size is smaller than 47 kD as demonstrated using cell cultures. The toxicity increases as the size of polyglutamine increases (Hackam et al., 1998).

7.3.1 Autophagy in the mouse brain

The implication of mutant huntingtins accumulation have been studied on mouse clonal striatal neurons. The cytoplasmatic vacuoles or inlusions developed in the cytoplasm causing the cells to shrink (Kegel et al., 2000). These vacuoles contained cathepsin D which is a marker for lysosomes suggesting the involvement in the endosomal-lysosomal pathway even under normal conditions. However, the amount of cathepsin D was elevated in the huntingtin-positive cells. The elevated activation of endosomal-lysosomal pathway might be viewed as the response to the presence of mutant huntingtin, allowing its removal and restoring homeostasis (Kegel et al., 2000).

Indeed, there were early as well as late autophagosomes observed in the FLAG-huntingtin-positive vacuoles suggesting the induction in autophagy. Some autophagosomes matured into autolysosomes but the induction was not sufficient to prevent neuronal degradation (Kegel et al., 2000).

The mutant huntingtin aggregates are visible only in the axons of striatal projection neurons contributing to neurodegeneration in HD knock-in mice. The huntingtin CAG repeats are largely expanded in HD mice, compared to controls. The neuropil aggregates composed of degenerated axons are present in the axonal terminals probably also containing some degenerated mitochondria (Li et al., 2001). However, some studies suggest that the presence of mutant huntingtin has no significant effect on the autophagy. The ratio of LC3-I to tubulin was alike when compared to htt-130Q cells in the brains from HD mice. Autophagy was not effective in reducing the huntingtin aggregates as demonstrated with rapamycin induction. Rapamycin treatment only increased the LC3 levels compared to control brains but the aggregates remained in the neurons. The levels of LC3 in the cerebellum, striatum and cortex in 2,4 and 24 months old HD mice did not change suggesting that autophagy was not induced or suppressed before the development of huntingtin aggregates (Li et al., 2010).

7.3.2 Autophagy in the human brain

The aggregates of mutant huntingtin with the NH2-terminal fragment were detected in the postmortem brain tissue of HD patients in contrast to control brain samples. The aggregates promote neuronal degeneration contributing to the development of Huntington's disease (DiFiglia et al., 1997).

The induction of autophagy using rapamycin was a useful treatment for the clearance of huntingtin aggregates. The total number of aggregate-containing cells was reduced compared to the striatal cell cultures that had no rapamycin treatment (Ravikumar et al., 2002). These findings suggest that the induction of autophagy might be an effective treatment of Huntington's disease. To further support these findings, the mutant huntingtin associated with autophagosomes in clonal striatal cells (x57 cells) increased as the autophagy decreased. The mutant huntingtin fragments were also reduced when rapamycin was applied (Qin et al., 2003).

The amount of aggregates significantly increased when the autophagy inhibitor 3-MA was applied to the neuronal cells. 3-MA prevented the formation of autophagosomes and therefore the aggregates could not be cleared using this pathway. The increased amount of aggregates leading to neuronal death was observed even upon the addition of N^6,N^6-dimethyladenosine (DMA) inhibitor (Ravikumar et al., 2002). When bafilomycin A1 (BAF) was applied to striatal cells, the maturation of autophagosomes was precluded, contributing to the development of huntingtin aggregates (Qin et al., 2003).

7.4 Therapy

The impact of autophagy regulation have been described in many studies investigating its role in neurodegenerative diseases. The induction of autophagy was successful in the clearance of the aggregated proteins in many cases.

Rapamycin is an effective mTOR inhibitor but other inhibitors can be used such as rapamycin analog temsirolimus that also inhibits mTOR and stimulates autophagy (Siracusa et al., 2017). Another rapamycin analog everolimus is also effective in mTOR inhibition (Dubois et al., 2014). Activators of AMPK such as metformin can be used to induce autophagy as the amount of autophagosome-positive cells increased in mice treated with metformin compared to controls (Buzzai et al., 2007).

IP3 inhibitors independent of mTOR, such as carbamazepine, could stimulate autophagy and increase LC3-II levels in the hippocampes of AD mice improving their memory capacity and spacial learning (Zhang et al., 2017).

However, the therapeutic treatment by these substances is still unclear as the mTOR inhibitor Torin 1 induced autophagy which contributed to neuronal death (Malagelada et al., 2010).

8. Conclusion

In conclusion, autophagy is a highly significant degradation process that can be exploited as a target for treating neurodegenerative diseases. The therapy should be aimed at restoring basal autophagy that maintains the homeostasis and is able to prevent neuronal damage (Hara et al., 2006). The common trait of the discussed neurodegenerative diseases is the aggregation of proteins that are toxic for the neuronal cells. The oxidative damage contributes to neuronal death reducing the number of neurons in specific brain areas. The damage and loss of neurons is the major cause of developing physical and psychological symptoms.

In Alzheimer's disease, the role of autophagy is not clear. Some studies suggest that induced autophagy might contribute to the development of senile plaques composed of β-amyloid (Aβ) peptide and tau protein causing neurodegeneration. However, autophagy can also elevate LC3-II levels and effectively remove the protein aggregates under certain conditions (Yu et al., 2005). Indeed, there is a neuronal death in many brain regions under the suppression of autophagy as the aggregates are not fully removed (Hara et al. 2006).

In Parkinson's disease, the chaperone mediated autophagy seems to be attenuated suggesting that other degradation systems might be activated (Yang et al., 2009). Macroautophagy can be induced removing α-synuclein in the substantia nigra protecting the neurons from degeneration (Yu et al., 2009). Indeed, mitophagy is impaired in PD as damaged mitochondria are not fully eliminated using this pathway (Gatt et al., 2016).

In Huntington's disease, autophagy is induced removing mutant huntingtin, but the degradation system is not efficient enough to prevent neurodegeneration (Kegel et al., 2000). On the other hand, some studies suggest that the presence of mutant huntingtin has no effect on autophagy (Li et al., 2010).

Nowadays, we cannot establish any cause and effect relationship between autophagy and neurodegeneration. The mechanism of those processes should be studied further in more detail to enable development of a more effective therapy for treating neurodegenerative diseases.

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