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REVIEW ARTICLE

Role of cathepsins in Alzheimer's disease: A systematic review

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Alzheimer's disease (AD) is a multifactorial disease. In addition to the precipitating of two proteins beta-amyloid peptide and neurofebrillary tangles, which are the main mechanisms involved in the pathogenesis of AD, other factors such as inflammatory mechanisms and changes in lysosomal enzymes play an important part in the pathogenesis of this disease. Increased and decreased lysosomal proteases, such as cathepsin, can lead to functional impairment and gradual death of neurons. The aim of this review was to investigate the role of cathepsins in the pathogenesis of AD. To conduct this review, relevant articles published between 2000 and 2016, and indexed in reliable databases including PubMed, Google Scholar, Scopus and Web of Science were retrieved. After reviewing the articles, 30 articles that directly addressed the subject of this review were included in final analysis. Cathepsins exacerbate intracellular conditions in neurons, by processing beta-amyloid precursor protein and converting it into amyloid beta. They also play a protective role against AD and fight it by catalyzing the decomposition of beta-amyloids and converting them into the cut out forms of the carboxyl C-terminus. In addition, the 24 kDa fragment resulting from the effect of cathepsin D on apolipoprotein E (ApoE) is the second binding to the receptor in the ApoE. This fragment may also be the cause of the pathogenicity of Apo E in AD. Identifying and explaining the mechanisms involved in the pathogenesis of AD can play a significant role in the prevention and treatment of this disease. Since cathepsins play a pivotal role in the decomposition of beta-amyloid and reduction of the risk of AD, further studies can be considered an effective approach to study AD.
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INTRODUCTION

Recent studies have shown that switching off and activating the proteolytic endosomal-lysosomal system can contribute to the pathophysiology of certain inflammatory diseases (Dickinson, 2002; Leichsenring *et al.*, 2008; Conus and Simon, 2010). Enzymatic proteolysis is accomplished by the nucleophilic attack of enzymes on carbonyl, followed by general acid and base hydrolysis (Garcia-Touchard *et al.*, 2005; Doucet and Overall, 2008).

In the human genome, more than 569 proteolytic enzymes are found, thus comprising the second largest family of enzymes in humans. Extracellular proteolysis is important for many biological processes such as tissue alteration, wound healing, and processes development.

On the other hand, extracellular proteolysis plays an important role in pathophysiologic processes such as cancer, chronic inflammatory diseases, cardiovascular diseases such as atherosclerosis and restenosis, activation of precursor proteins (Masoudi *et al.*, 2015; Khayeri *et al.*, 2016) (including pro-enzymes and prohormones), antigen presentations at the cell surface by major histocompatibility complex (MHC) II, rearrangement of bone, keratinocyte differentiation, hair follicle cycle, apoptosis, arthritis, Parkinson's disease, and AD (Roshangaran and Masoudi, 2016; Masoudi *et al.*, 2017).

Generally, in diseases caused by lysosomal enzymes, there is an impairment of brain development, retardation, or mental disorders, and the presence of

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intracellular protein deposits is one of the characteristics of this type of disorders, as with AD (Noormohammadi *et al.*; Masoudi *et al.*, 2013).

Studies have also shown an increase in the levels of cathepsins B and D in the brain lysosomal system of patients with AD in the early stages of the disease. After being synthesized in the endoplasmic network and passing through post-translational modifications via two receptors, CD-MPR and CI-MPR, cathepsins enter the lysosomal system, mainly the primary endosomal vacuoles containing the Rab-5 marker. In the early stages of AD, the levels of cathepsins B and D increase inside the primary endosomes containing the Rab-5 marker due to the increased CD-MPR receptor expression. In addition, in the brain tissues of AD patients, the levels of cathepsin B, and possibly cathepsin D, have been observed to increase, representing the increased expression of these two enzymes genes.

However, the experimental studies using the RT-PCR technique have shown that in the peripheral system of patients, including peripheral blood lymphocytes and fibroblasts, there is a reduction in transcription in cathepsin D (Nixon *et al.*, 2005; Urbanelli *et al.*, 2008). Regarding the role of cathepsins C and D in AD, there are controversial arguments and evidence whose general focus is on the involvement of these two enzymes in the emergence or prevention of AD.

These two enzymes are capable of being involved in the beta-amyloid precursor protein (βAPP) processing and amyloid beta production. Perhaps the reason for increase in the levels of beta amyloids in the lysosomal system and especially the primary endosomes in the early stages of the disease, with the increasing activity of the lysosomal system, including the increase in utophagy and endocytosis, along with increase in the levels of lysosomal enzymes, especially cathepsins B and D, is the direct or indirect effect of these cathepsins in processing the βAPP to produce beta amyloid.

These two cathepsins *in vivo* have beta- and gamma-secretase activity compared to the βAPP models (Urbanelli *et al.*, 2008; Chen *et al.*, 2010). The prevailing argument is that the breakdown of beta-amyloids, except for certain specific pathological conditions, plays a major role in these proteolytic enzymes, and the beta-amyloids produced throughout the endocytosis and phagocytosis processes may be degraded after being transported to lysosomes by the proteolytic enzymes, and particularly the cathepsins, present in them, which indicates the role of cathepsins B and D in clearing amyloid plaques and developing AD(Mueller-Steiner *et al.*, 2006).

Therefore, with respect to available evidence and significant role of cathepsins in the pathogenesis of AD, the aim of this review was to investigate the role of cathepsins in the pathogenesis of AD.

To conduct this review, relevant articles published between 2000 and 2016, and indexed in reliable databases including *PubMed*, *Google Scholar*, *Scopus* and *Web of Science* were retrieved. The articles without abstract and full text in English were excluded from the study. After reviewing the articles, the articles (n: 30) that directly addressed the role of cathepsins in AD were included in final analysis.

RESULTS

The lysosomal system mainly comprises a family of intracellular components that are permanently linked to each other. This system includes more than 80 types of acid hydrolases, including cathepsins. Cathepsins degrade most of the proteins entering the lysosomal system rapidly into their amino acid components.

However, chemical and enzymatic post-translational modifications throughout the aging process and certain diseases yield proteins that are more resistant to degradation so that they are not completely degradable or are partly degraded. These proteins accumulate as a result of this abnormality. Cathepsin is one of the enzymes that are released into the bloodstream and cellular fluids under stress. Cathepsins represent highly susceptible lysosomal proteases that play an important role in lysosomal apoptosis and induce metastasis in the extra-

cellular matrix.

Cathepsin is a biomarker for many diseases including cancer, diabetes, keratoconus, and epilepsy. Table 1 shows the relationship between catepsin and its role in the pathogenesis of various diseases (Lalioti *et al.*, 2003; Alakurtti *et al.*, 2005; Määttä *et al.*, 2006; Masini *et al.*, 2009; Las and Shirihai, 2010).

Generally, doctors may use a certain number of common laboratory tests, such as vitamin B12, T4, thyroid-stimulating hormone, complete blood cell count, electrolyte test, the erythrocyte sedimentation rate, HIV antibody, the rapid plasma reagin, drug screen, as well as computed tomography (CT), magnetic resonance imaging (MRI), (Tau/Aß42), Apo E, PS1, PS2, and amyloid precursor protein (APP) genotype to diagnose and differentiate AD from other causes of amnesia (Table 2).

The cathepsins are divided into three groups based on the amino acids of their active sites: a) cysteine cathepsins (B, S, K, V, F, L), b) aspartate cathepsins (E, D), and c) serine cathepsins (G)(Garnero *et al.*, 1998; Sambrano *et al.*, 2000). Cathepsins are active in acid pH and are produced and synthesized in the form of zymogen called procathepsin, where the propeptide of the N-terminal is isolated either as enzymatic or via the autocatalytic separation of propeptides, and activated under acidic conditions and is converted to mature cathepsin through the autoproteolytic pathway (Turkington, 1992; Yang *et al.*, 2013).

In total, the substrates and the necessary materials enter the lysosomal system via two pathways: 1. Heterophagy including receptor-mediated endocytosis, pinocytosis and phagocytosis for transporting extracellular materials into lysosomes; 2. autophagy

Table 1: Cathepsin as a biomarker in a variety of diseases

Role	Results
Cathepsins B and D in diabetics	Transcription of lysosome-associated membrane protein 2 and cathepsins B and D decreased in diabetics (Masini et al., 2009).
Cathepsins B and D in type 2 Diabetes	They observed that autophagy in beta cells was activated after induction of stress as a protective mechanism. However, the increase in observed autophagosomes in diabetic patients and in beta cells under treatment with free fatty acids can reflect a decrease in autophagic flux due to decreased lysosome formation and cathepsin (Las and Shirihai, 2010).
Cathepsin in the development of epilepsy	The genes involved in idiopathic generalized epilepsy; this gene is located on the long arm of chromosome 21, and consists of three short exons and two introns. The coded protein by the cystatin B (CSTB) gene is distributed among most cells and tissues of the body and the cysteine inhibitor is the proteases of the cathepsin B or 3 family; if the mutation of this gene and the increase in cathepsin B occur, this type of epilepsy occurs (Lalioti et al., 2003).
Cathepsin in the development of epilepsy	The unstable expansion of minisatellite in the promoter region of the CSTB gene is the most common mutation in patients with equine protozoal myeloencephalitis and leads to a decrease in the level of mRNA and an increase in cathepsin B, and thus development of epilepsy (Alakurtti et al., 2005).
Cathepsin in the development of epilepsy	Point mutation c.212A>C in the exon 3 of the cystatin B gene, which leads to the replacement of glutamine with proline and reduction in the binding of cytosine B to cathepsin B, and thus epilepsy (De Haan et al., 2004).
Cathepsin in the development of keratoconus	The total protein in the cornea is reduced. Theoretically, a small increase in the quantities of degenerative enzymes and a reduction in the quantities of proteinase inhibitors can lead to degeneration of the stroma extracellular matrix. Levels of degenerative lysosomal enzymes, such as esterase and acid phosphatase, and those of the matrix metalloproteinases in the cornea, such as cathepsins G and B, increase in patients with keratoconus (Sawaguchi et al., 1989; Zhou et al., 1998; Määttä et al., 2006).

Table 2: The tests used for differential diagnosis of Alzheimer's disease from other forgetfulness causing diseases (Nixon, 2007)

Test type	Test	Sample	Usage	Application
Imaging techniques	Computed tomography (CT)	Full-body scan	Differentiation of Alzheimer's disease from other disorders, diagnosis of Alzheimer's disease in advanced stages	Stroke and cerebral atrophy (Brain degeneration in the final stages of Alzheimer's disease)
Imaging techniques	Magnetic resonance imaging (MRI)	Full-body scan	Differentiation of Alzheimer's disease from other disorders, diagnosis of Alzheimer's disease in advanced stages	Stroke and cerebral atrophy (Brain degeneration in the final stages of Alzheimer's disease)
Less common laboratory tests	Association between amyloid beta and Tau protein (Tau/Aß42)	Cerebrospinal fluid	Helping differentiate Alzheimer's against other types of amnesia	In patients with symptoms, decreased levels of amyloid beta 42, accompanied by an increase in Tau protein levels, indicates an increased risk of developing Alzheimer's disease, regardless of the cause of the disease.
Less common laboratory tests	Genotype Apo E	Blood	Genotyping ApoE genotype and conducting complementary tests to confirm Alzheimer's disease diagnosis or rule out the likelihood of its development	Apolipoprotein E (ApoE)/e4 increases the risk of Alzheimer's disease at an early age in symptomatic patients. The variations of the e2 and e4 alleles are also associated with dyslipidemia.
Less common laboratory tests	PS1	Blood	Genetic mutation test (There are only a few laboratories)	Considered the cause of the early onset of familial Alzheimer's disease in about half of the cases.
Less common laboratory tests	PS2	Blood	Genetic mutation test (There are only a few laboratories)	Familial Alzheimer's disease, a mutation observed in a limited number of families
Less common laboratory tests	APP	Blood	The genetic mutation test is still being studied and is not clinically available.	Familial Alzheimer's disease, a mutation observed in a limited number of families

for transporting intracellular materials into lysosomes. Both of these pathways are associated with the processing of βAPP and the pathogenesis of AD. Amyloid beta is typically produced in the autophagic and endocytotic processes (Nixon *et al.*, 2005; Urbanelli *et al.*, 2008; Kokjohn and Roher, 2009; Lee *et al.*, 2010) (Figure 1).

In autophagic pathway, βAPP-containing organelles and in the endocytic system, extracellular materials along with the cytoplasmic membrane of endosomes are the major sources of βAPP. In this case, the beta -amyloid produced by lysosomes is efficiently degraded. If the endocytotic process is reduced or accelerated, the amount of produced amyloid beta proportionately increases or decreases as well (Bhojak *et al.*, 2001).

Dynamic and active changes in the contents of the substrates, the hydrolyses and the internal pH, between autophagosomes and endosomes, provides suitable conditions, depending on the cell health, for both the production and the breakdown of beta -amyloid, and therefore in both of these pathways (autophagy and endocytosis), beta-amyloid is normally produced. But, notably the beta-amyloid produced in this pathway are mainly degraded, after being transported to lysosomes, by cathepsins, which have the adequate specificity of decomposition for amyloid beta.

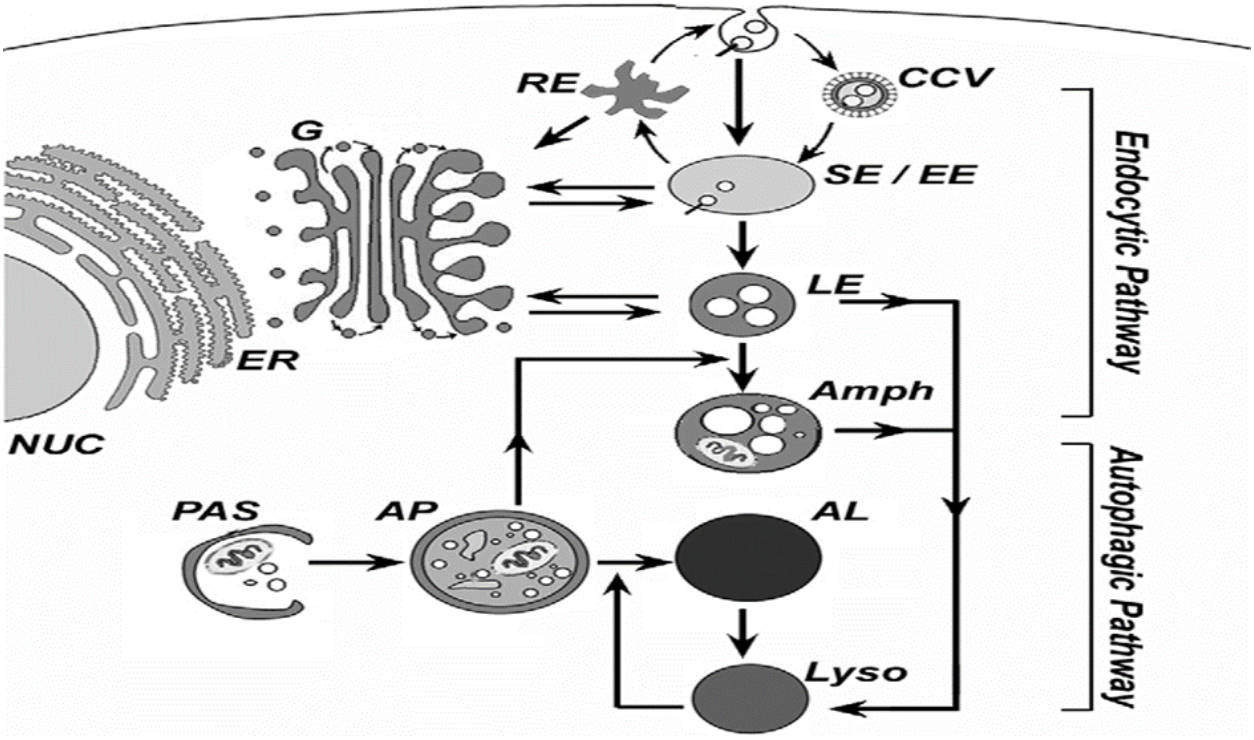

Figure 1: A schematic representation of the lysosomal system in the brain that represents the pathways of beta-amyloid precursor protein processing in endosomes and autophagosomes (Riemenschneider *et al.*, 2006). PAS: Pre-Autophagic Structure; AP: Autophagosome; AL: Autophagolysosome; LE/MVB: Late Endosome/Multivesicular Body; EE: Early Endosomes; SE: Secondary Endosomes; LE: Late Endosomes.

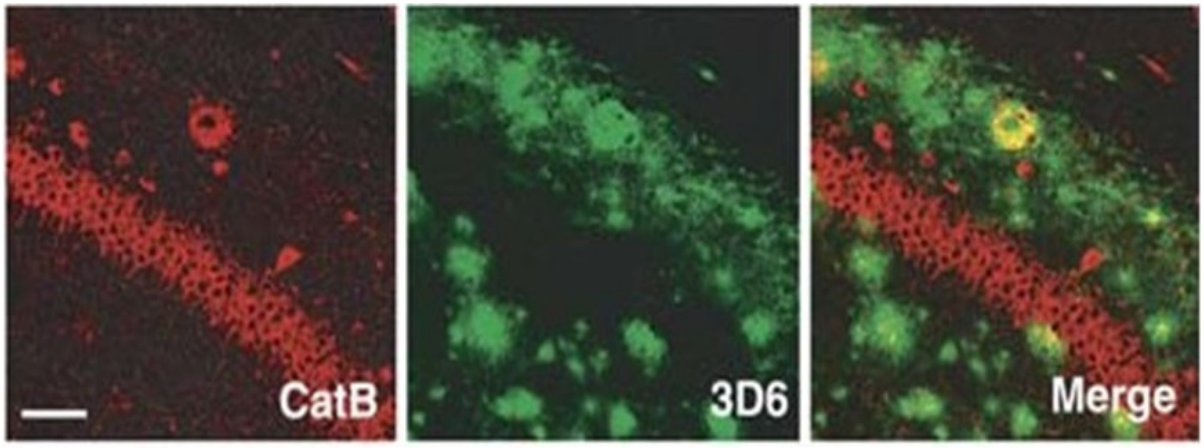

Figure 2: Co-accumulation of cathepsin B and amyloid beta in amyloid plaques in brain tisdues of transgenic mice aged 16-20 months with human beta-amyloid precursor protein (Mueller-Steiner *et al.*, 2006), (A): Immunostaining against cathepsin B with Cat B antibody makes the tissue section red; (B): Immunostaining against beta-amyloid by anti-amyloid veta antibody 3D6 makes the tissue green; (C): The yellow signal in the integrated A and B Figure illustrates the presence of both amyloid beta and cathepsin B inside the plaque (Mueller-Steiner *et al.*, 2006).

Generally, beta amyloid levels are determined by the balance between production and degradation, and any defect in the proteolytic degradation of this peptide can contribute to the pathogenicity and pathogenesis of AD (Perry *et al.*, 1987; Zhou *et al.*, 2006; Chen *et al.*, 2010). Two Alzheimer's disease-associated pathways-heterophagy and autophagy-are illustrated in Figure 2 (Nixon, 2007).

DISCUSSION

Some achievements suggest that the gene expression and production of cathepsin D multiply in AD, which is due to neurolysis and increased concentration of this protease in the cerebrospinal fluid (Mueller-Steiner *et al.*, 2006; Riemenschneider *et al.*, 2006; Majumdar *et al.*, 2007; Lee *et al.*, 2010; Sundelöf *et al.*, 2010). Chen *et al.,* (2010) showed that an increase in blood cholesterol levels in humans is a predisposing factor for AD. They argued that increased cholesterol levels in the endosomal system affected the structure and function of lysosomes.

In these experiments, it has been shown that the specific activity of cathepsin D and the acid phosphatase in the lysosomal system decreases due to an increase in cholesterol levels. Changes in cholesterol hemostasis can increase the production of beta amyloid in the brain, but it does not specify the mechanism and the extent to which circulating cholesterol can affect beta -amyloid levels as well as the spreading of pathology in AD. One mechanism for entering cholesterol into receptor-dependent endothetic neurons. As the level of cholesterol in the circulation increases with the change in the integrity and the hemostasis of the brain-blood barrier, the amount of cholesterol entering the brain tissue increases as well. As a result of this increase, cholesterol-dependent cholesterol-derived endostiosis increases in neurons.

Along with the endocytotic process, some of the cytoplasmic membrane of the neurons also enters the cells as endosomal vacuoles, which themselves contain some βAPP, resulting in a higher amount of βAPP substrate entering into the endosomal-lysosomal system. This endosomal-lysosomal system thus turns into a place where there is necessary secretases for converting βAPP to beta-amyloid, and therefore the production of amyloid beta will increase.

In addition, the increase in cholesterol levels in the endosomal system affects the structure and function of lysosomes, which reduces the specific activity of cathepsins D, B, and E and acid phosphatase (ACP) in the lysosomal system. This matter may also be the reason for the mechanism of AD in sporadic cases of this disease due to increased cholesterol (Chen *et al.*, 2010).

Other studies have shown that cathepsin D can contribute to the pathogenesis of AD by degrading ApoE. Although the exact mechanism of the role of ApoE in AD is unknown, new findings have led to the suggestion that the remaining residues from proteolytic degradation of ApoE may be involved in the pathogenesis of this disease. Aspartic proteases in the lysosomal system of the brain cells seem to be involved in ApoE proteolytic activity. Cathepsins D and E are two types of these aspartic proteases. ApoE proteolytic activity is related to either of these two cathepsins. The involvement of these two cathepsins has been demonstrated in the proteolysis of the ApoE derived from human purified cathepsins D and E with two types of ApoE, consisting of lipid-free neo-compound ApoE3 and ApoE4 as well as human ApoE4 lipoped with dipalmitoyl phosphatidyl cholin (DPPC) (Zhou *et al.*, 1998).

Cathepsin D on both ApoE3 and ApoE4 lacked lipid and the ApoE4 with lipid exhibited the same degradation effect. The dominant product in this process is a 24 kDa fragment that is similar to that obtained from the brain tissues of AD patients. However, cathepsin E only exerts effect on ApoE3, which is free of lipid, and does not affect lipid-containing ApoE4. This is despite the fact that the proteolysis-derived pattern of cathepsin E on the lipid-free ApoE4 and the ApoE3 is different from the pattern of its degradation by cathepsin D that is similar to the tissue pattern of patients. Regarding these results, it seems that cathepsin E is more likely to contribute to the degradation leaing to the ApoE pathogenesis in the brain of AD patients, compared with cathepsin D.

On the other hand, histological studies performed on the sections of AD patients suggest the simultaneous accumulation of cathepsin D and ApoE in a series of senile plaques and Tangles. There is also a positive correlation between the duration of the disease and the percentage of these plaques. Additional results have suggested that the 24 kDa fragment resulting from the effect of cathepsin D on ApoE is the second binding to the receptor in Apo E. The cause of the pathogenicity of ApoE in AD may also be the same (Zhou *et al.*, 2006).

In addition to the above evidence, it has been shown that the levels of the proteolytic lysosomal enzymes present in the microglia are higher than those in the macrophages of the J774 macrophages. Microglia are capable of scavenging beta-amyloids by means of scavenger receptor type A (SRA), but due to their near-neutral pH (about 6) inside their lysosomes, these lysosomal enzymes, in particular, cathepsins, fail to provide enough activity to break down beta-amyloids in microglia. In the pathologic sections of the brain tissues of AD patients, the beta-amyloid-containing microglia are frequently found around amyloid plaques. It seems that if the pH in the lysosomes of the microglia resches more favorable levels, the beta-amyloid proteolytic decomposition will be accomplished more optimally.

In experimental models, the use of inflammatory agents, such as interleukin 6 and macrophage colony stimulating factor, allowed microglia to decompose beta-amyloid. It can also be said that the mutation in the PS1 gene causes lysosomal disorders, and in the event of mutations or the mutations leading to decreased activity or deletion of the PS1 gene, certain impairments of the lysosomal system occur, including structural disorders such as enlargement and accumulation of lysosomal mediators, impaired acidity of the endosomes and autophagosomes, as well as the decreased activation of cathepsin D, as the main lysosomal aspartic protease.

Although the pathological effect of these mutations in AD has been frequently reported to be the production of amyloid beta from its precursor, βAPP, but in this regard, it is important to note that this effect is not observed in all diseases that occur due to a mutation in PS1, so in this case, with respect to AD, in addition to increasing amyloid beta production, loss of another function or the other specific and biological functions of the PS1 can be one of the most important causes of the onset of the disease. Experimentally, the elimination of the PS1 gene results in complete loss of autophagy, while this effect has the least possible effect on lysosome-independent proteolytic systems.

Studies have determined that the role of PS1 in the process of lysosomal proteolysis is to assist the acidification process of the internal contents of the components of the lysosomal system. Naturally, for the internal contents of the endolysosomes and autophagolysosomes to be acidic, the system requires an energy-dependent proton pump called vacuolar [H +] ATPase (V ATPase). The acidification of the internal contents of these lysosomal structures is an important process in their maturation, as well as in the activation of proteolytic enzymes such as cathepsins L, D, and B. This proton pump consists of a membrane complex called V0 and a cytosolic complex called V1, each of which in turn consists of several subunits.

After synthesizing the subunits in the endoplasmic network and completing the post-translational processes on them, the subunits are transported to the endosomes and autophagosomes and are placed in their cell membrane, thereby performing the task of acidifying the endolysosomes and the autophagolysosomes. Studies have shown the role of PS1 in completing the post-translational modification on the V0a1 subunit of this pump for n-glycosylating it in the endoplasmic network.

The V0a1 subunit, after completing the translation process in the endoplasmic network, quickly contacts with an enzyme in the endoplasmic membrane called the oligosaccharyltransferase (OST) via physical interaction with the PS1. By doing so, a part of the V0a1 protein structure is in appropriate contact with the active site of the OST. It has been shown that in the case of mutation or deletion of the PS1 gene, this process is impaired or not performed and

thus V0a1 cannot be placed in the membrane of lysosomal structures. The absence of this subunit in the membrane of lysosomal structures, in turn, leads to the formation of the V-ATPase complex, and as a result of the acidification of the inner contents of lysosomal vacuoles, their maturity is confused.

If the process of acidification of these structures is not carried out, cathepsin will not be activated for several reasons. For example, the acidification of lysosomal structures is necessary for separation of CI-MPR from cathepsins. If the lysosomes are not acidic, these receptors will still remain bound to the cathepsins and will not allow them to be activated.

On the other hand, the environment should be acidic for the full maturity of proteolytic cathepsins. Apart from these cases, as repeatedly mentioned in this article, for the maximum function of cathepsins, there is a need for an acidic environment. Whether it is possible to examine the changes in the pattern of cathepsins for the diagnosis and assessment of different stages of AD is still unclear and many studies have been carried out in this regard.

CONCLUSION

In general, it can be concluded that beta-amyloid degradation, except for certain specific pathological conditions, represents the major role of cathepsin, and the beta-amyloid produced throughout the endocytosis and phagocytosis are degraded by cathepsin after being transported to the lysosomes. The specific activity of cathepsin D and the acid phosphatase in the lysosomal system decreases due to increased cholesterol levels. This may also be the reason for the mechanism of AD in the sporadic cases of this diseases due to high blood cholesterol levels. In addition, the 24 KD fragment resulting from the effect of cathepsin D on ApoE contains a second binding to the Apo E receptor. This fragment may be the reason for the pathogenicity of Apo E in AD.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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