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EDITORIAL

Cathepsin D as a therapeutic target in Alzheimer's disease

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1. Introduction

Several neurodegenerative diseases are characterized by the accumulation of ubiquitin-positive protein aggregates in affected brain regions [1]. These misfolded/aberrant proteins are toxic for neuronal function and contribute to neurodegeneration. Protein quality control, including autophagy and proteasome system, is necessary for the removal of aggregated proteins that are harmful for the brain. Autophagy-lysosomal system plays crucial roles in both normal cellular homeostasis and disease states. Indeed, the constitutively active autophagy is critical for post-mitotic cells, such as neurons that cannot simply dilute harmful molecules through cell division [2]. The lysosomal system consists of communicating acidic compartments which contain over 80 lysosomal hydrolases, including proteases, nucleases, phosphatases, sulfatases, lipases, and glycosidase. Lysosomal cathepsins can be divided into three groups: cysteine (cathepsins B, C, F, H, K, L, O, S, V, W, and X), aspartic (cathepsins D and E), and serine (cathepsin G) proteases [3]. The aspartic cathepsin D and some of the cysteine cathepsins are ubiquitous and among the most abundant lysosomal proteases. The acidic environment of the lysosomal lumen, resulting from the action of the vacuolar H^+-ATPase, facilitates the degradation process by loosening the structures of macromolecules and is optimal for the activity of lysosomal hydrolases [3].

In the Alzheimer's disease (AD) brain, progressive abnormalities of the endosomal-lysosomal system, such as increase in size and volume of early endosomes, are a prominent neuropathological feature [2]. Amyloid beta (Aβ) peptide has also been detected in these enlarged endosomes that are immunopositive for the early endosomal marker rab5 [4]. Indeed, several studies have identified the endosomal-lysosomal pathway as an important regulator of the processing of amyloid precursor protein (APP) [5]. Early endosomes produce Aβ from APP in normal cells and mediate the uptake of Aβ and soluble APP. The upregulation of the lysosomal system occurs in vulnerable cell populations and results in increased numbers of lysosomes with elevated expression of lysosomal hydrolases [6]. As AD pathogenesis progresses, lysosomal dysfunction appears to occur with the build-up of vacuolar structures and the accumulation of Aβ. The degeneration of the compromised neurons leads to the release of these structures into the extracellular space, where they associate with deposits of Aβ.

2. Cathepsin D in the brain of Alzheimer's disease

Cathepsin D (CTSD) is a soluble lysosomal aspartic endopeptidase coded by the *CTSD* gene and synthesized in the rough endoplasmic reticulum as pre-proCTSD. After removal of signal peptide, the proCTSD is targeted to endosomes to form an active, 48-kDa, single-chain intermediate, and then to the lysosomes to form the fully active mature protease, composed of a 34-kDa heavy chain and a 14-kDa light chain. No endogenous inhibitors of CTSD are known and natural inhibitors, called pepstatins, are synthesized by *Streptomyces* bacteria, but not by eukaryotic animal cells [7]. The mature CTSD is predominantly active at pH below 5, found in the lysosomes. However, CTSD is the only proteolytic enzyme whose expression, in different tissues, is regulated in response to growth factors, cytokines, and vitamins [7]. Due to its ability to cleave structural and functional proteins and peptides, CTSD plays numerous physiological functions in the cells including metabolic degradation of intracellular proteins and the activation of enzymatic precursors [3,7,8]. In the central nervous system, CTSD is particularly important in the control of neuronal homeostasis, cell migration, and interneuron communication. CTSD-mediated proteolysis is essential to neurons by accomplishing the degradation of unfolded/oxidized protein aggregates that continuously reach the lysosomes via autophagy or endocytosis [7,9]. Many proteins produced in neurons are physiologic substrates of CTSD and would abnormally accumulate if not efficiently degraded (e.g. the amyloid precursor (APP), α-synuclein, and huntingtin) [5,7,10]. Therefore, dysfunctions of CTSD into the lysosomal system are closely related to mechanisms of neurodegeneration (Figure 1).

Nixon's group was the first to demonstrate the abnormal immunoreactivity of CTSD in senile plaques of AD patients, thus suggesting a molecular link between aberrant CTSD and AD [4,6]. Other studies also demonstrated higher protein levels of CTSD and the upregulation of its gene expression in the hippocampus of MCI patients [11]. The most obvious role of CTSD in AD is its involvement in the clearance of Aβ and tau proteins through the autophagy-lysosomal system. Hence, altered lysosomal function, as demonstrated in AD brain extract and *in vivo* models of the disease, could lead to the accumulation of Aβ and tau proteins and to the formation of senile plaques an tangles [7]. In agreement with these observations, disturbances

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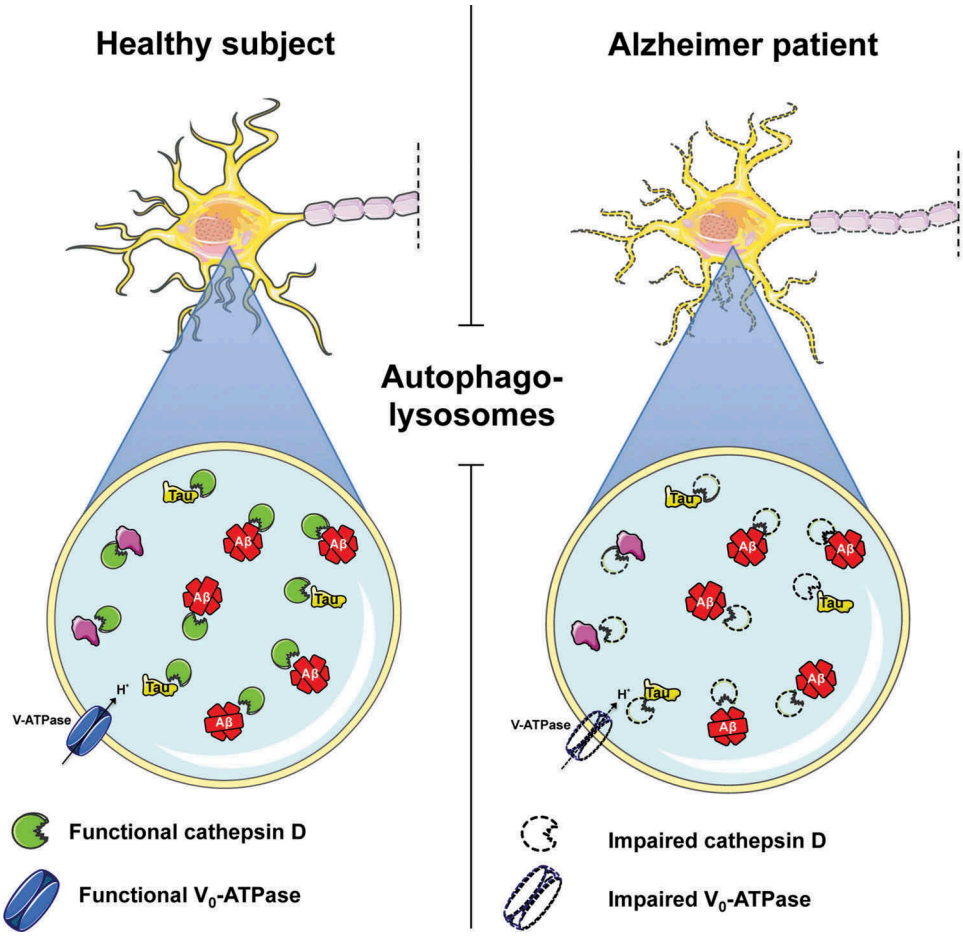

Figure 1. Role of CTSD in the processing of AD pathogenic proteins (APP and tau) and autophagy. Proteolytic activity of CTSD may contribute to pathogenic APP processing by favouring the cleavage of APP into Aβ fragment. As well, lack of CTSD may lead to increased production of a truncated (fibrillar-prone) form of tau. Notably, CTSD is essential for lysosomal integrity by acting in concert with other acidic proteases to degrade misfolded/damaged proteins including Aβ and Tau.

in the expression of active CTSD and in autophagy-lysosomal proteolysis have been reported in the frontal cortex of young Down's syndrome (DS) patients before and after the evident manifestation of AD-like symptoms [9,12,13]. In this scenario, the upregulation of CTSD expression in AD may represent a compensatory protective mechanism to counteract the deficit of the neurons of eliminating protein aggregates [9,11]. Noteworthy, CTSD has also been implicated in the processing of APP, apolipoprotein E, and the tau protein [5,10,14], all important factors of AD pathogenesis. It was demonstrated that the ablation of CTSD favors the formation of a truncated neurotoxic form of tau, and that the overexpression of CTSD could represent a protective response [5].Noteworthy, *in vitro* studies suggested that CTSD may possess a Beta-site amyloid precursor protein cleaving enzyme 1-like activity being able to cleave APP, thus resulting in increased Aβ production. Nonetheless, this process is highly efficient on mutant (Swedish mutation) APP but not on wild-type APP [5,10]. However, these findings have not been confirmed in *in vivo studies*. Experimental evidence also demonstrates that CTSD is involved in the processing of ApoE and it is well established that the presence of the ApoE4 isoform associates with increased risk for AD [14].

Variants of *CTSD* gene might impede the proper functions of proteolytic degradation, thus increasing the risk of AD. A CTSD amino acid change (Ala38-to-Val) increases pro-CTSD secretion and alters intracellular maturation [8]. It has been proved that this polymorphism was associated with the level of Aβ and tau, even if there was a significant heterogeneity in the results obtained by different studies [8].

Intriguingly, we found that CTSD is oxidatively modified in DS brain compared with healthy controls and that this modification results in a slight increase of enzyme activity [9]. This is interesting because DS neuropathology has many common features of AD, including deposition of plaques and tangles very early in life. In parallel, we also found the oxidation of Vo-ATPase (vacuolar H^+-ATPase) suggesting the impairment of lysosomes acidification and protease activity. To date, these data support oxidative stress-induced lysosome permeabilization that can lead to translocation of cathepsins in the cytoplasm and eventually its inappropriate activation. Moreover, increased activity of CTSD may be a compensatory response to partially disturbed autophagy and/or and attempt to upregulate autophagic flux in response to defective autophagosomal maturation, as suggested in AD neurons [15].

3. Expert opinion

Accumulating evidences suggest that dysfunction of protein quality control—proteasome and lysosomal system—is a prominent feature of degenerating neurons that is associated with accumulation of oxidized/misfolded proteins in pathological brain. Lysosomal proteases, in particular the cathepsins, play important functions including processing and activation of a wide range of proteins and hormones, lysosomal death pathway, autophagy, aging, and other processes. However, proteases catalyze irreversible cleavage of peptide bonds and their activities must be finely regulated.

Abnormalities in the lysosomal pathway have been demonstrated to occur early in AD pathology, before robust deposition of neurofibrillary tangles or senile plaques. Intriguingly, some studies reported the involvement of CTSD in the processing of APP, apolipoprotein E, and the tau protein, all crucial pathogenic events in AD. Since APP undergoes extensive proteolytic processing, it is not surprising that lysosomal proteases have been associated with AD; however, APP catabolism involves several other factors.

The picture that emerges from experimental evidences on the role of CTSD in AD suggests that (i) increased expression of CTSD may be a compensatory mechanism to restore lysosomal function and (ii) knockout of CTSD favors aberrant generation of a C-terminally truncated (fibrillary-prone) form of tau iii) CTSD may be susceptible to oxidative modifications in diseased brain, such as DS, that results in its impaired activity.

In the brain, CTSD is crucial for neuronal homeostasis and CTSD-mediated proteolysis is essential for the clearance of unfolded/oxidized proteins that are delivered to lysosomes to prevent their toxic accumulation. However, considering that the functionality of vacuolar H^+-ATPase, which is essential for the maintenance of acid environment of lysosome, i.e. activity of CTSD, is affected in AD brain, a combined approach may be required to achieve restoration of CTSD to brain physiological levels.

In conclusion, we suggest that targeting CTSD in AD may require a better understanding of key regulatory mechanisms that contribute to impair its activity that may ultimately result in failed protein degradation and aging-dependent neurodegeneration.

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Declaration of interest

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