The roles of the proteasome pathway in signal transduction and neurodegenerative diseases

Jiao-Jiao CHEN, Fang LIN, Zheng-Hong QIN

Department of Pharmacology and Laboratory of Aging and Nervous Diseases, Soochow University School of Medicine, Suzhou 215123

Abstract: There are two degradation systems in mammalian cells, autophagy/lysosomal pathway and ubiquitin-proteasome pathway. Proteasome is consist of multiple protein subunits and plays important roles in degradation of short-lived cellular proteins. Recent studies reveal that proteasomal degradation system is also involved in signal transduction and regulation of various cellular functions. Dysfunction or dysregulation of proteasomal function may thus be an important pathogenic mechanism in certain neurological disorders. This paper reviews the biological functions of proteasome in signal transduction and its potential roles in neurodegenerative diseases.

Keywords: proteasome; signal transduction; protein misfolding; neurodegenerative disease

1 Introduction

The integrity of cellular processes depends on the proper balance of synthesis and degradation of cellular proteins. Controlling protein half-life by degradation has emerged as a main cellular mechanism to maintain cellular homeostasis. There are two major degradation pathways involving either a collection of proteolytic enzymes within the lysosome or the multicatalytic proteolytic core of the ubiquitin-proteasome system (UPS). The UPS degrades intracellular proteins with a tag of polyubiquitin chain. In addition to protein degradation, the UPS also plays important roles in diverse physiological functions and pathogenesis in many diseases. Abnormalities of UPS have been found in certain neurodegenerative diseases and cancers. This review focuses on the roles of UPS in signal transduction and neurodegenerative diseases.

2 Characteristics of the ubiquitin-proteasome

pathway

2.1 Molecular organization of 26S proteasome In eukaryotic cells, most proteins in the cytosol and the nucleus are degraded via the ubiquitin-proteasome pathway. The 26S proteasome is a 2.5-mDa molecular machine consists of about 31 different subunits, which catalyzes protein degradation^[1]. It contains a barrel-shaped proteolytic core complex (the 20S proteasome), capped at one or both ends by 19S regulatory complexes (19S cap, PA700), which recognize ubiquitinated proteins^[2-4]. The regulatory complexes are also involved in unfolding and translocation of ubiquitinated proteins into the interior of the 20S complex, where ubiquitinated proteins are degraded into oligopeptides. The catalytic 20S core particle is only able to degrade unfolded substrates, excluding native, folded proteins. Structural assembly and enzymatic mechanism of the 20S complex have been well-documented. It has been proposed that two subunits that compose of the 20S particle in the arch-ad-bacterium are the ancestors of the different α and β subunits and the basic architecture of the proteasome consists of four rings of seven subunits each^[5,6], whereby the two outer rings are composed of α subunits and the two inner rings of β subunits ($\alpha\beta\beta\beta\beta\alpha$). Electron tomographic studies laid the basis for our current knowledge of

Corresponding author: Zheng-Hong QIN
Tel: 86-512-65880406
Fax: 86-512-65880406
E-mail: zhqin5@hotmail.com
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the structural assembly of the 20S proteasome^[7]. It is a barrel-shaped complex with three cavities, whereby the two outer cavities are jointly formed by one α and one β subunit ring and the inner cavity is formed by the two β rings. Based on this intriguing structure and because the proteasome degrades target proteins with high specificity, the proposal was made that degradation of the substrate proteins after unfolding occurred in the inner cavity of the proteasome barrel^[8]. This prediction has been proven to be correct^[9]. The structural and genetic studies have identified the proteasome as an N-terminal nucleophile (Ntn)-hydrolase with threonine as the N-terminal active nucleophile^[10-12]. *In vitro* studies have led to the purification of two high molecular mass proteases of 20S and 26S whereby the latter species was able to degrade ubiquitinated lysozyme^[4,13,14]. The 20S protease was shown to be actually a subcomponent of the ATP-dependent 26S proteasome. But the functional organization of the 19S complex is less well understood. Most subunits of the 19S complex have been identified; however, specific functions have been assigned to only a few of them. A low-resolution structure of the 26S proteasome is obtained by electron microscope, but the precise arrangement of subunits in the 19S complex is unclear^[15].

2.2 Enzymes of the ubiquitination cascade There are three different sets of enzymes termed E1, E2, and E3, shuttling ubiquitin and linking ubiquitin to the protein substrates. E2 and E3 or their different combinations recognize each substrate's unique degradation signal, thereby conferring exquisite specificity for ubiquitination of diverse protein substrates^[16].

2.2.1 E1 (ubiquitin activating enzymes) This enzyme generates a high-energy thiolester intermediate with ubiquitin that involves an internal Cys residue.

2.2.2 E2s (ubiquitin carrier proteins or ubiquitin conjugating enzymes, UBCs) The activated ubiquitin is transferred from E1 to a Cys residue of an E2 enzyme, thereby generates yet another thiolester intermediate. Many E2s have been identified, at least 13 in yeast (UBC1-13) and more than 20 in mammals, reflecting their role in conferring the specificity for ubiquitination of diverse protein substrates. All E2s contain a conserved about 130 amino acid-long catalytic core sequence, i.e. UBC domain. Some E2s are involved in specific cellular processes while the role of others is still obscure. However, they all act via their function by UBCs and all are inactivated by mutation of the active Cys residue.

For example the yeast UBC2/RAD6 is involved in degradation of "N-end rule" substrates and also in DNA repair. The mechanism that underlies this activity is still unknown. Another one is UBC3/CDC34, which is required for G1→S transition, probably via degradation of certain cell cycle regulators, while UBC4 and UBC5 are involved in the degradation of many short-lived, normal and abnormal proteins. Due to the specific effects of certain E2s on defined processes, it has been proposed that they can interact directly with the substrate proteins. While such interactions have been described using protein-protein interaction screening methods, their physiological significance is not clear. Most probably, the specific functions of E2s are due to their association with distinct E3s^[17].

2.2.3 E3s (ubiquitin-protein-ligases) An E3 enzyme is defined as a protein that binds the target substrates, either directly or indirectly, via ancillary proteins, and catalyzes transfer of ubiquitin from a thiolester intermediate on E2 or E3 to an amide linkage with the substrate or with a polyubiquitin chain already anchored to it^[18]. Since the target proteins bind to the ligases prior to conjugation, E3s are key players in determining the high specificity of the system. Despite their importance, the number of known E3s is few and the information concerning their mode of action is rare. Lack of sequence homology among different E3s and the frequent association of these enzymes with multisubunit complexes in which the identity of the ligase subunit is not known, render their study difficult.

2.3 Proteolysis Proteolysis is pivotal for cellular and developmental regulation. Due to its irreversible nature, proteolysis is ideally suited for regulating unidirectional pathways such as cell cycle progression or differentiation^[19]. In eukaryotes, selective proteolysis is largely mediated by the ubiquitin/proteasome system. Early studies have indicated that this pathway is divided into two steps: substrate recognition, which is brought about by the ubiquitin conjugation system, and degradation, catalyzed by the 26S proteasome. Unexpectedly, various additional proteolytic factors that are neither typical E2 or E3 enzymes nor components or regulators of the proteasome are also involved.

Some scientists have confirmed that the COP9 signalosome (CSN) is a multiprotein complex of the ubiquitin-proteasome pathway. CSN is typically composed of eight subunits, each of which is related to one of the eight sub-

units that form the lid of the 26S proteasome regulatory particle. It is widely accepted that CSN directly interacts with cullin-containing E3 ubiquitin ligases. In addition to its interaction with E3s, CSN may also regulate proteolysis by its association with protein kinases and de-ubiquitylating enzymes.

Scientists have suggested that polyubiquitination is not necessary for proteasome-mediated degradation through elucidating the mechanism for proteolysis of ornithine decarboxylase (ODC). ODC is an enzyme whose degradation is mediated by its binding to the cofactor antizyme 1 (AZ1). Once bound, ODC-AZ1 can be recognized by the proteasome, and ODC is degraded in an ubiquitin-independent manner. However, it is unclear whether the means by which AZ1 promotes degradation differs fundamentally from that of polyubiquitin chains. A recent study demonstrated that ODC-AZ1 competes with substrate-linked and free polyubiquitin chains for the occupancy of the same binding site on the proteasome. Thus, the mechanism for the degradation of ODC seems to represent a specialized evolutionary adaptation that closely mimics ubiquitination.

The proteasome degrades cytosolic proteins as well as proteins that fail to pass protein quality control in the endoplasmic reticulum (ER). Misfolded proteins in the ER are recognized by ER-specific E3 ligases that mediate polyubiquitination of the misfolded protein on the cytosolic side of the ER and are subsequently degraded by the proteasome^[20].

However, two recent important advances in the field were the discoveries of non-proteolytic functions for ubiquitin: activation of transcription, and the discovery of ubiquitin-like proteins. Some of the latter proteins function through the covalent modification of their targets, and are involved in numerous non-proteolytic functions such as directing proteins to their subcellular destination and protecting other proteins from ubiquitylation. Others have different functions in the ubiquitin signaling system that do not involve the covalent modification of target proteins. Other interesting discoveries include the finding that the ubiquitin-like protein Apg12 is essential for the function of the vacuole in autophagy in *Saccharomyces cerevisiae*^[21], and that ubiquitylation functions as a signal for sorting proteins into endosomes^[22] and the multiple vesicular body (MVB) pathway^[23]. The latter sorting pathways are complex and involve

the modification of both the cargo substrates and the components of the vesicular system. These discoveries closed an exciting historical cycle, linking together what were thought to be two distinct systems—lysosomal digestion and modification by ubiquitin and ubiquitin-like proteins. All these studies have led to the emerging realization that this mode of covalent conjugation has a key role in regulating—through both proteolytic and non-proteolytic mechanisms—a broad array of cellular processes, which include the cell cycle and cell division, cell growth and differentiation, the activation and silencing of transcription, apoptosis, immune and inflammatory responses, signal transduction, receptor mediated endocytosis and the sorting of proteins in the cell, various metabolic pathways, and protein quality control. As there are numerous substrates that need to be targeted and processes that have to be regulated, it is not surprising to discover that aberrations in the system are implicated in the pathogenesis of many diseases, including several malignancies and neurodegenerative disorders^[24].

2.4 Ubiquitin-molecular kiss of death Ubiquitin is composed of 76 amino acid residues and its primary sequence is highly conserved from yeast to mammals, thus resulting in essentially identical tertiary structure. X-ray diffraction and proton NMR analyses have allowed the determination of the tertiary structure of ubiquitin, revealing its highly compact and rigid nature: only the four carboxy terminal amino acid residues, including Gly-76 that is activated in an ATP-dependent manner for subsequent ubiquitin ligation with the ϵ -amino group of an internal Lys residue of the protein substrate. Another important residue, Lys-48, of ubiquitin is located on the surface of a Type III reverse turn, providing its ϵ -amino group for an easy access to form an additional amide isopeptide bond to generate multi ubiquitin chains.

2.5 The ubiquitin-proteasome system cascade The ubiquitin-proteasome pathway degrades diverse cellular proteins with exquisite specificity. To accomplish this, highly modular and elaborate ubiquitination machinery is employed to recognize specific substrates^[25]. In the first step of the ubiquitin conjugation cascade, the carboxy group of Gly-76 of ubiquitin is activated by ubiquitin-activating enzyme (E1). This step involves the hydrolysis of ATP to PPi to generate an ubiquitin adenylate intermediate bound to an E1 enzyme. Subsequently, an active site Cys residue of E1 covalently links to ubiquitin via a high-energy thioester linkage, with the concomitant

release of AMP. Following activation, activated ubiquitin is then transferred by transacylation reaction to a thiol group of an active site Cys residue of E2 (ubiquitin carrier protein or ubiquitin-conjugating enzyme). Finally, E2 shuttles ubiquitin either directly to a protein substrate by itself or in cooperation with ubiquitin-protein ligase (E3), to form an amide isopeptide bond between the carboxyl group of Gly-76 of ubiquitin and a ϵ -amino group of the protein substrate's internal Lys residue. The last step occurs by first transferring ubiquitin from E2 to E3, which accepts ubiquitin in a similar thiol linkage and then to the protein substrate. In some cases, however, covalent linkage between E3 and ubiquitin is not observed and it appears that ubiquitin is directly transferred from E2 to the protein substrate in a ternary E2-E3-substrate complex.

Once the protein substrate is mono-ubiquitinated, a polyubiquitin chain is formed through the same ubiquitination conjugation cascade, as described above, in which the carboxyl group of the carboxy terminal Gly-76 of ubiquitin is covalently linked to an internal Lys residue of ubiquitin that is already conjugated to the protein substrate.

It should be emphasized that the specificity of ubiquitination is largely determined by a series of E3 enzymes and E3 multiprotein complexes, and is specific to one or a few corresponding protein substrate(s) and E2 enzymes, each of which is dedicated to their cognate E3 enzyme(s). As a result, different combinations of E2 and E3 enzymes allow selective tagging and degradation of specific intracellular proteins. In contrast, there is a single family of E1 that is highly conserved.

3 Proteasome mediated intracellular signal transduction

In addition to degradation of cellular proteins, several intracellular signal transduction systems are controlled or regulated by the ubiquitin-proteasome.

3.1 Nuclear factor-kappa B-dependent signaling The transcription factor nuclear factor-kappaB (NF- κ B) is a regulatory protein, whose activation is controlled by the ubiquitin-proteasome pathway. Originally thought to be a B cell-specific transcription factor, NF- κ B is a ubiquitous and central player in a variety of cellular processes, including immune and inflammatory responses, apoptosis, and cell proliferation [26-28]. NF- κ B exists in different forms and each form is

composed of various members of the Rel family protein, reflecting the complexity of its response to diverse stimuli. In resting cells without extracellular stimuli, NF- κ B is held latent in the cytoplasm as a complex bound with unphosphorylated inhibitor κ B (I κ B), thereby blocking the nuclear translocation of NF- κ B. In response to the proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1), IKK is activated. The later in turn phosphorylates the critical Ser residues in the regulatory domain of a member of the I κ B family. Phosphorylation of κ B by IKK leads to subsequent degradation of κ B. As a result, free NF- κ B is allowed to translocate into the nucleus and activates transcription of NF- κ B target genes.

Extensive study of such signal-induced degradation of κ B has revealed that the ubiquitin-proteasome pathway plays a crucial role in NF- κ B activation. Specifically, it is found that a variety of inducing signals converges on IKK α and β , which eventually phosphorylate highly conserved Ser-32 and Ser-36 in I κ B α [29,30]. These two highly conserved Ser residues reside in the amino terminal regulatory domain that precedes a well-conserved ankyrin core and a PEST domain. IKK β phosphorylates I κ B, resulting in its ubiquitination by the SKP1-CUL1-F-box ligase containing the F-box protein β TrCP (SCF- β TrCP) [31-33] ubiquitin-ligase complex and subsequent degradation by the proteasome. The SCF complex contains the RING-domain protein ROC1/RBX1, which binds to UBC4/5, allowing this E2 to ubiquitinate I κ B at two conserved N-terminal Lys residues Ser-32 and -36. The polyubiquitinated I κ B remains associated with NF- κ B, but is selectively degraded by the 26S proteasome, while NF- κ B itself is spared. It should be noted that other members of I κ Bs, such as I κ B β and ϵ in mammals, also share a similar amino terminal regulatory domain that contains two invariant Ser residues within a defined region. Their consensus sequences are believed to undergo a similar signal-induced degradation mediated by the ubiquitin-proteasome pathway.

Interestingly, the ubiquitin-proteasome pathway is also implicated in controlling the activity of NF- κ B pathway through a limited proteolysis of p105 [34,35]. Full-length p105 forms a stable complex with p65 [36-38], in a similar manner as a ternary complex formation of p50/p65 dimer bound with I κ B. Analogous to recognition of I κ B α , the SCF complex containing the F-box protein β -TrCP recognizes p105, albeit two different recognition motifs are used for I κ B and p105. While

an exact mechanism for p105 processing in response to NF- κ B inducing stimuli is yet to be determined, results are beginning to shed light on the importance of IKK-mediated phosphorylation of p105 that leads to accelerated processing of p105^[39]. Thus, pathways involving cotranslational degradation by the 26S proteasome of newly synthesized p105 appear to provide an alternative mechanism to control the activity of NF- κ B via a balanced production of p50 and p105 in the cells^[40].

3.2 In caspase signaling Mitochondria apoptotic pathway is mediated by cytochrome c (Cyt c) release and caspase activation. When death-inducing signals reach the mitochondria, a series of cell death related events mediated by mitochondria take place. These cell death related events are mediated by mitochondria, namely the permeability transition (PT) of the inner membrane, the loss of the inner mitochondrial membrane potential, and Cyt c release collectively bring the cell to a point of no return in the apoptotic pathway. Once Cyt c is released into the cytoplasm, it forms a complex with ATP, apoptosis activating factor-1, and procaspase-9 called the apoptosome, which cleaves procaspase-9 to caspase-9^[34]. The caspase-9 then cleaves procaspase-3, its immediate downstream substrate, initiating the cascade of protease activation in the execution phase of apoptosis.

In addition to the mitochondria, one of the key regulators of lymphocyte apoptosis is the proteasome^[35], as a large proteolytic multienzyme complex residing in the cytoplasm and the nucleus. Some investigators have previously observed that the inhibition of proteasome activity results in an inhibition of caspase-3 activity. This finding suggests that the proteasome may exert a regulatory role upstream in cell death events.

In detail, many of the critical biochemical and morphological changes occurring during apoptosis are brought about by activation of caspases, and caspase activity is regulated partly by the inhibitor of apoptosis (IAP) family of proteins, such as XIAP, c-IAP-1 and c-IAP-2, and also by Smac/DIABLO (second mitochondrial activator of caspases) and Omi/Htra2^[33]. The IAP proteins, particularly XIAP, are very potent inhibitors of some caspases, such as caspase-3, -7 and -9. However XIAP, c-IAP-1 and -2 also contain a C-terminal RING domain and act as E3 ubiquitin protein ligases, regulating the degradation of several proteins as well as themselves^[32]. During apoptosis, caspase activation resulted in

the cleavage of three specific subunits, S60, S5a and S1, the 19S regulatory subunits of the proteasome. Two of these subunits, S60 and S5a, are involved in the recognition of polyubiquitinated substrates in the proteasome and S1 is involved in holding together the lid and base of the 19S regulatory complex. Caspase-mediated cleavage of these proteasomal subunits resulted in an inhibition of both ubiquitin dependent and independent substrates, which could facilitate apoptosis by providing a feed forward amplification loop^[41]. However, this would clearly depend on the balance of pro- and antiapoptotic molecules to be degraded by the proteasome and the relative availabilities and affinities of the various E2 and E3 enzymes for these different substrates and whether any of these was affected during apoptosis. Inactivation of the proteasome following caspase-mediated cleavage may disable the proteasome, thus interferes with its role in the regulation of key cellular processes, thereby, facilitates the induction of apoptosis.

3.3 The effect of proteasome on apoptotic pathways Numerous cell proteins involved in signal transduction pathways that control apoptosis are degraded by 26S proteasomes. A key role for the ubiquitin/proteasome system in the regulation of apoptosis has been proposed^[42]. Regulatory molecules involved in apoptosis, such as p53, proapoptotic Bcl-2 family members, including Bax, Bak, Bid, and Bik are proteasomal substrates^[43]. The proteasome plays an important role in maintaining homeostasis by removing such potentially harmful molecules. The other functions of proteasome in the process of apoptotic are regulating the cell cycle, as some cell cycle regulators are important players in apoptosis.

The data obtained in tumor cells demonstrate the key role of 26S proteasomes in maintaining neoplastic proliferation, and show that the 26S proteasome proteolytic activity performs the survival function only in rapidly growing and cycling cells. The proteasome is required for cells in survival and normal progression through the cell cycle. Inhibition of the cellular proteasome activity in G1 can inevitably trigger programmed cell death.

Proteasome inhibitors have been found to induce apoptosis in several types of neoplastic and rapidly growing cells (including cells of the hemopoietic origin)^[44], and proteasomes have been suggested to perform only the antiapoptotic function in neoplastic cells. Alterations in homeostasis by various cellular stressors that prevent protein

folding cause an accumulation of misfolded proteins in the ER, which causes ER stress. Proteasome dysfunction together with other unknown mechanisms could contribute to protein aggregation throughout the life-time. Down-regulation of chaperones induction along with impaired protein degradation, could lead to protein accumulation. Importantly, accumulation of misfolded proteins in ER. ER stress leads to activation of an apoptotic pathway involving the up-regulation of CHOP (the C/EBP homologous protein)/GADD153 and the activation of caspase-12^[45-48]. When protein overloading in ER by proteasome dysfunction, it will produce ER-stress and finally the activation of an apoptotic pathway which links with CHOP and caspase-12.

4 Proteasome dysfunction and neurodegenerative diseases

The pathologic states associated with the UPS can be classified into two mechanism-based groups: those that result from a mutation in a UPS enzyme or a target substrate (loss of function), leading to stabilization of certain proteins, and those that result from accelerated degradation of the target protein (gain of function). This section presents several well-studied examples of aberrations in the UPS that are directly linked to human diseases. The two mechanisms maybe both exist in the etiology of the neurodegenerative diseases^[49]. Because the perturbations in ubiquitin-proteasome-mediated proteolysis lead to pleiotropic effects on neurons including cell dysfunction, degeneration and cell death. One of the effects to be important is protecting cells against the toxic effects of misfolded and unfolded proteins by proteasome. Proteasome-dependent degradation clears misfolded proteins from the cytosol, the nucleus, and the endoplasmic reticulum (sometimes), whereas chaperones bind to exposed hydrophobic regions in proteins, thus preventing aggregation and enabling protein for folding. If a given protein does not acquire a properly folded conformation in a reasonable time frame, it becomes eligible for degradation. Several regulatory mechanisms can help to coordinate this partitioning.

The accumulation of insoluble protein deposits in the affected cells is a common feature of many age-related neurodegenerative disorders, such as tauopathies (e.g. Alzheimer's and Pick's disease), synucleinopathies (e.g. Lewy body disease) and polyglutamine diseases (Huntington's disease, and several spinocerebellar ataxias). In addition, in

non-neuronal disorders inclusions are present as well (e.g. alcoholic liver disease, inclusion body myositis and α -antitrypsin deficiency). Although the deposits vary in protein composition, shape and localization, each of these structures (e.g. aggregates, aggresomes or inclusions) is mainly composed of insoluble misfolded proteins (e.g. huntingtin, α -synuclein or hyperphosphorylated tau), different molecular chaperones (e.g. heat shock proteins) and various components of the ubiquitin-proteasome (e.g. E3 ligases such as CHIP, 19S and 20S proteasomal subunits of the 26S proteasome).

4.1 Alzheimer's disease and UPS The substantial role of the UPS in Alzheimer's disease (AD) pathology is increasingly recognized. For example, proteasome activity was found to be lower in AD brains than in age-matched controls. In addition, high levels of ubiquitin were detected in brain homogenates and cerebrospinal fluid samples of AD patients. Intriguingly, tau and A β , the two major players in AD pathology, as well as the mutant form of ubiquitin, UBB+1, were found to alter proteasome activity. These findings strongly support the relevance of altered proteasomal degradation in AD. Tau is a microtubule-associated protein of about 55 kDa. Besides playing a role in the stabilization of axon microtubules, tau has also been shown to interact with the actin cytoskeleton and plasma membrane and play a role in neurite outgrowth, enzyme anchoring and intracellular vesicle transport regulation^[50]. In AD, tau is thought to be dissociated from microtubules because of its hyperphosphorylation, which probably affects axonal transport. Hyperphosphorylated and abnormally folded tau accumulates in neurons as paired helical filaments (PHF), leading to neurofibrillary tangles (NFT) and eventually ghost-tangles. More than 25 potential phosphorylation sites have been identified in PHF-tau isolated from AD brain^[51-53]. The tau protein normally exists as an unfolded protein and was suggested to be degraded by the 20S proteasome *in vitro*, both from the N to C and from the C to N terminus^[54]. This implies that tau, just like other unfolded proteins, can be degraded by the 20S proteasome in an ubiquitin-independent manner. Meanwhile, some new studies demonstrate that tau, both phosphorylated and non-phosphorylated, is a substrate of ATP/Mg²⁺-dependent proteasome^[55]. Extracellular deposition of amyloidogenic proteins is the main neuropathological feature in AD and prion-related encephalopathies. Numerous studies indicate a causal role of these aggregated proteins, β -amyloid (A β) and prion

protein, in the development of the respective diseases. The possibility of a common etiology for these diseases based on protein misfolding is controversial and recent findings seem to exclude a role of protein aggregation in the onset of the disease though it may become essential for its complete development. It has been proposed that aggregation of different proteins directly impairs the function of the ubiquitin-proteasome system which is likely to be involved in the positive feedback induced by the protein accumulation that altered its function, which in turn increased the production of aggregated proteins.

A study that presents a possible mechanism for indirect proteasome inhibition by A β demonstrates that the toxicity of extracellular A β in neuronal cell lines is mediated by the E2 ubiquitin-conjugating enzyme E2-25K/Hip2^[56]. E2-25K expression was increased upon A β treatment, and antisense E2-25K cDNA abolished A β -induced toxicity. These findings seem to be biologically relevant, as the E2 enzyme was also found to be up-regulated in AD brain.

Many oxidized proteins and lipid peroxidation products accumulate in AD brains, suggesting that oxidative stress is an important pathogenic event in AD^[57]. The exact cause for oxidative stress in AD brain remains unclear. A β is likely a contributor, as A β generates free radicals through metal-catalyzed reactions that result in neuronal death^[58]. Free radicals peroxidize membranes lipids and oxidize proteins. In general, such oxidized proteins are degraded by the proteasome. A relationship between oxidative stress and proteasome function is supported by the finding that the familial form of amyotrophic lateral sclerosis (ALS) is associated with a mutation in the superoxide dismutase (SOD) gene^[59]. Increased levels of oxidatively modified proteins and a parallel increase in proteasome activity were observed in cell lines that stably express a mutant form of human SOD^[60]. These cells were more sensitive to proteasome inhibition, which caused accumulation of neuronal nitric oxide synthase (nNOS) and programmed cell death. The proteasome activity of these cells was, most likely, up-regulated to keep nNOS levels down and prevent oxidative stress-induced cell death. In a different study, proteasome inhibition in cells expressing a mutant SOD form caused the formation of aggregates containing ubiquitinated or nitrated α -tubulin, SOD and α -synuclein. The presence of oxidized proteins in AD is probably related to proteasome dysfunction.

4.2 Parkinson's disease and UPS Parkinson's disease (PD) is one of the most severe and widespread age-related neurodegenerative disorders, affecting almost 3% of the population aged over 65. It is characterized by inclusions named Lewy bodies. The cellular and molecular events underlying PD pathogenesis are still poorly understood. PD appears to be a multifactorial pathology in which factors such as oxidative stress and mitochondrial dysfunction, may lead to the selective degeneration of dopaminergic neurons in substantia nigra.

Functional analysis of parkin as E3 leads to the clear conclusion that AR-JP is caused primarily by abnormality of cellular protein metabolism due to dysfunction of parkin in dopaminergic neurons. It is also clear that loss of function parkin mutations cause accumulation of the target(s) that is not ubiquitinated, and that reduced proteasomal elimination of this parkin substrate(s) might ultimately cause death of dopaminergic neurons.

In PD, classic Lowy body (LB) have a relative restricted distribution, which corresponds to dopaminergic neurons of the substantia nigra and ventral tegmental region, noradrenergic neurons of the locus coeruleus, catecholamine cells of the medulla oblongata, serotonergic neurons of the raphe nuclei, and specific cholinergic neurons. In general, classic LBs involve monoamine neurons and appear as roundish structures made up of a hyaline core and a pale peripheral halo. The latter, at ultrastructural level corresponds to filamentous material forming fibrillary elements which are characterized by the presence of the protein α -synuclein^[61].

In addition to classic LB, in PD there are also atypical LB, which are called "cortical LB" since they occur in telencephalic brain regions. Both typical and cortical LB at light microscopy own a pale eosinophilic structure which stains for thioflavin-S. Nowadays, characterization of LB is grounded on immunohistochemistry since they are labeled by antibodies against ubiquitin, α -synuclein, parkin, synphilin-1, torsin A and other proteins belonging to a multienzymatic pathway named the ubiquitin proteasome system. In addition, they contain various chaperone proteins like heat shock proteins and other molecules that play an important role in the physiology of the cell growth such as cyclins. In addition, LB can be labeled for typical fibrils and other cytoskeletal proteins

An important component of Lewy bodies is α -synuclein,

a presynaptic protein. Point mutations in the α -synuclein gene have been linked to familial PD, suggesting a critical role for α -synuclein in PD pathogenesis. The presence of α -synuclein, ubiquitin, and other proteasomal subunits in Lewy bodies suggested the involvement of α -synuclein and a dysfunction of proteasomal degradation pathway in PD pathogenesis^[62]. Whether α -synuclein is a substrate for proteasome, however, remains controversial since both positive and negative data have been reported^[63]. Nonetheless, α -synuclein accumulation or aggregation can form various complexes by interacting with numerous proteins and modulate cellular functions including proteasomal degradation pathway^[64]. Both monomeric^[65] and aggregated α -synucleins selectively bind to the proteasomal protein S6V, a subunit of the 19S cap. Furthermore, impairment of α -synuclein function may perturb the ubiquitin-dependent degradation of α -synuclein-associated proteins such as synphilin^[66] or tyrosine hydroxylase^[67] resulting in alterations in cellular functions^[68].

Heterozygous mutations in the gene encoding ubiquitin carboxy-terminal hydrolase L1 (UCHL1) have been causally implicated in families with PD^[69], although the data are contentious^[70]. UCHL1 might function not only as an ubiquitin hydrolase (hydrolyzing ubiquitin chains to free ubiquitin monomers) but also as an ubiquitin ligase^[71]. The role of UCHL1 in human PD is still unclear, but loss of function is known to be deleterious, as recessive deletion of part of this gene causes gracile axonal dystrophy in mice. Although this phenotype is not obviously parkinsonian, it is associated with the formation of ubiquitinated intraneuronal aggregates^[72].

4.3 Huntington's disease and UPS Huntington's disease (HD) is one of fourteen known conditions caused by a CAG trinucleotide repeat expansion that is translated into a polyglutamine (polyQ) tract in the disease protein. This group of diseases includes HD, spinocerebellar ataxias types 1, 2, 3, 6, 7 and 17, Kennedy's disease and dentatorubral-pallidoluysian atrophy. Genetic and transgenic data suggest that the primary effect of these CAG/polyglutamine expansions is to confer a toxic gain-of-function on the mutant protein.

Nowadays, evidence has been provided that polyQ is a poor substrate for eukaryotic proteasome^[73]. A long polyQ stretch or a stable polyQ polar zipper hairpin^[74-76] may enter the catalytic core of the proteasome and occupy or block access to the proteolytic sites for an extended period of time. Some evidence suggests that when one site of the proteasome

is occupied by a substrate or an inhibitor, alterations occur at other sites^[77-79] provide evidence that occupancy of the caspase-like site activates the trypsin-like activity and inhibits the chymotrypsin-like activity. Recent studies have found that inhibiting substrate and inhibitor access to that site, the trypsin-like site could be activated and the chymotrypsin-like activity inhibited. The results show, with good consistency, that there are fundamental alterations of the UPS in Q111 [StHdh (Q111/Q111)] cells compared to Q7 cells [StHdh (Q7/Q7)] which all are the cell lines of model HD express full length wild-type or mutant huntingtin (htt) at endogenous levels. Alterations of the proteolytic function of the proteasome core were shown clearly. Altered degradation of firefly luciferase UPS reporters was also shown suggesting alterations in UPS processes preceding proteolysis in the proteasome core. The study shows fundamental alterations of UPS function in this cell model of HD^[80].

The mechanisms by which mutant htt causes disease remain elusive but it has been hypothesized that the UPS is involved in many aspects of HD pathogenesis^[81,82]. Htt is a substrate of the UPS and ubiquitylated mutant htt accumulates in cells^[82,83]. Aggregates are a pathologic hallmark of HD and many other neurodegenerative diseases. Neurons of HD patient brains are encumbered with neuronal intranuclear inclusions containing aggregated mutant htt^[83,84]. Recent evidence suggests that aggregates may be less toxic and that smaller forms of mutant htt such as misfolded monomers, oligomers, and fibrillar structures are the more toxic species. Although there remains much debate as to the toxic mutant htt species, there is significant evidence suggesting involvement of the UPS in formation and clearance of all forms of mutant htt^[85-87]. Thus, the UPS is likely to play a key role in the pathogenesis of HD. Further, it has been suggested that mutant htt expression results in immunoproteasome induction^[88,89], a proteasome made up of different isoforms of proteolytic subunits that results in a different profile of proteasome activity that is optimized for MHC II presentation of peptides.

Proteasome does clear mutant htt exon 1, but it is not clear if the proteasome clears soluble or aggregated protein. Some scientists demonstrated that the soluble mutant proteins with expanded glutamines can be degraded by the proteasome, but these proteins become resistant to proteasomal degradation when aggregated. The ubiquitin-proteasome

system may also play a role in the catabolism of full-length or larger htt fragments, as htt interacts with hE2-25K, a human E2 ubiquitin conjugating enzyme and is ubiquitinated. However, the residues that are ubiquitinated have not been systematically defined and it is not clear if ubiquitination occurs at different rates with full-length huntingtin compared to its various cleavage products.

Some scientists concluded that proteasome impairment is caused by aggregate formation. If proteasome function is impaired by mutant htt and other aggregates, then the degradation of many crucial regulatory short-lived proteins, such as transcription factors, would be impaired leading to cellular toxicity. Furthermore, some of the features of HD, like increased levels of oxidatively modified proteins/DNA, can be induced by proteasome impairment. They reported that decreased proteasome activity in cell lines expressing mutant htt was accompanied by cell death, activation of caspases 3 and 9 and disruption of the mitochondrial membrane potential. Both caspase activation and mitochondrial dysfunction occurred when the cells were treated with lactacystin, suggesting that such changes in HD models may be due to proteasome impairment. But these studies do not address if the proteasome inhibition in HD is a primary event, or if it is a consequence of other possibly earlier changes. Furthermore, we are not aware of publications that have tested if proteasome function is compromised in HD patient brains. It is also possible that mutant fragments *in vivo* do not induce the same responses as overexpression of short N-terminal huntingtin transgenes in cell models.

5 Future perspectives

What we can expect in the near future is the identification of an ever-increasing number of substrates and their specific E2 and E3 enzymes. It is hoped that this will solve some of the enigmas surrounding the mechanisms of specific recognition of the innumerable substrates of the system and the involvement of the UPS in human disease. For example, identifying the substrates of the parkin enzyme will shed light on the pathogenetic mechanisms that underlie certain forms of Parkinson's disease. The first drug in clinical use based on inhibition of the UPS is bortezomib, a proteasome inhibitor that blocks the entire ubiquitin system. The hope is to see the development of new, potent, and highly specific drugs that target the degradation pathways of a single or a

few proteins without affecting others. These novel drugs, which will mostly affect E3 enzymes, will have far fewer side effects than conventional chemotherapeutic agents. For example, drugs that inhibit the aberrant E3 (MDM2)-mediated targeting of the p53 tumor suppressor, which is observed in many malignancies.

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蛋白酶体在信号转导和神经退行性疾病中的作用

陈皎皎, 林芳, 秦正红

苏州大学医学院基础医学与生物科学学院药理教研室, 衰老与神经疾病实验室, 苏州 215123

摘要: 哺乳动物细胞内有两条蛋白降解途径: 泛素-蛋白酶体通路和自噬-溶酶体通路。蛋白酶体由多种蛋白亚单位组成, 在降解细胞短寿命蛋白中有重要作用。近来的研究发现蛋白酶体降解系统在细胞信号转导和细胞功能中有重要作用。蛋白酶体功能障碍或失调可能参与某些神经退行性疾病的致病机制。本文综述蛋白酶体在细胞信号转导中的生物学功能和在神经退行性疾病中的可能作用。

关键词: 蛋白酶体; 信号转导; 蛋白折叠异常; 神经退行性疾病