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Review

The ubiquitin proteasome system as a potential therapeutic target for treatment of neurodegenerative diseases

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Abstract. Impairment of "protein quality control" in neurons is associated with etiopathogenesis of neurodegenerative diseases. The worn-out products of cell metabolism should be safely eliminated *via* the proteasome, autophago-lysosome and exocytosis. Insufficient activity of these degradation mechanisms within neurons leads to the accumulation of toxic protein oligomers, which represent a starting material for development of neurodegenerative proteinopathy. The spectrum of CNS linked proteinopathies is particularly broad and includes Alzheimer's disease (AD), Parkinson's disease (PD), Lewy body dementia, Pick disease, Frontotemporal dementia, Huntington disease, Amyotrophic lateral sclerosis and many others. Although the primary events in etiopathogenesis of sporadic forms of these diseases are still unknown, it is clear that aging, in connection with decreased activity of ubiquitin proteasome system, is the most significant risk factor. In this review we discuss the pathogenic role and intracellular fate of the candidate molecules associated with onset and progression of AD and PD, the protein tau and α-synuclein in context with the function of ubiquitin proteasome system. We also discuss the possibility whether or not the strategies focused to re-establishment of neuroproteostasis *via* accelerated clearance of damaged proteins in proteasome could be a promising therapeutic approach for treatment of major neurodegenerative diseases.

Key words: Alzheimer's disease — Parkinson's disease — Tau protein — α-synuclein — Proteasome — Heat shock proteins

Abbreviations: AD, Alzheimer`s disease; aPTM, abnormal posttranslational modifications; APP, amyloid precursor protein; CHIP, carboxy terminus of Hsc70 interacting protein; CNS, central nervous system; D421, aspartate 421; E1, ubiquitin activating enzyme; E2, ubiquitin conjugating enzyme; E3, ubiquitin ligase; GDA, geldanamycin; GSK3β, glycogen synthase kinase-3 beta; HSF1, heat shock factor 1; Hsc70, hHeat shock cognate 71 kDa protein; Hsp, heat shock protein; LB, Lewy bodies; MTBD, microtubule-binding domain; NFTs, neurofibrillary tangles; PHF, paired helical filaments; PD, Parkinson`s disease; POMP, proteasome maturation protein; PROTACS, proteolysis targeting chimera molecules; REG, 11S regulatory particle; Rpn4, zinc finger transcription factor; SDS, sodium dodecyl sulfate; SNCA, alpha-synuclein gene; Ub, ubiquitin; UBA52, human ubiquitin fusion gene; UBB, polyubiquitin B precursor; UBB^{+1}, ubiquitin B mutated protein; UCHL1, ubiquitin carboxy-terminal hydrolase L1; UPS, ubiquitine proteasome system.

Introduction

Alzheimer's and Parkinson's diseases are the most common neurodegenerative diseases of advanced age, with distinct clinical symptoms. They are predominantly sporadic, meaning that in more than 95% of cases, there is no evident link to monogenic inheritance. Recently about 44.4 million people worldwide were diagnosed with Alzheimer's disease (AD) and 7–10 million with Parkinson's disease (PD), however, the number of patients is expected to double every 20 years. In 2050 there would be 115.38 million AD patients worldwide and 18.65 million AD in Europe (Prince 2013).

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Among the serious human diseases, AD and PD belong to those which still lack the causative, "diseases modifying" treatment that would slow down the pathological processes. The pathogeneses of AD and PD share several significant characteristics. Both diseases are influenced by various risk factors, with 'age' being the most prominent. On the molecular level, they both are classified as proteinopathies, characterized by increased accumulation of misfolded and cleaved proteins in the intracellular and extracellular space. The spectrum of proteinopathies is very broad and except of AD and PD includes Lewy body dementia, Pick disease, Frontotemporal dementia, Huntington disease, Amyotrophic lateral sclerosis and many others (Golde et al. 2013). A significant difference in clinical phenotypes of these disorders can be explained by the type of the affected neuronal populations within the brain and selective vulnerability of the neurons. While the characteristic dopaminergic deficit seen in PD is caused by degeneration of the substantia nigra, the cholinergic deficit evident in AD can be traced back to loss of neurons in the nucleus basalis of Meynert (Braak et al. 1995; Breydo et al. 2012).

It was described that degradation mechanisms, preferentially the ubiquitin proteasome system, plays one of the key roles in progress of AD and PD and probably also in their etiopathogenesis (Bedford et al. 2008). In recent literature a regulation of ubiquitin proteasome system (UPS) activity can often be considered as a promising strategy for the development of an efficient treatment of neurodegeneration (Dantuma and Bott 2014). Although this idea is broadly discussed, it is a matter of fact that the majority of compounds that regulate UPS activity are known for their toxicity and negative side effects.

Protein foldopathies and UPS – the major regulator of proteostasis in neurons

A common characteristic feature of many neurodegenerative disorders, including AD and PD, is the formation of higher molecular protein aggregates (Ballatore et al. 2007; Goedert et al. 2013). This is a pathognomic feature, however, experimentally is documented that it can be also pathogenic (Zilka et al. 2006). In Alzheimer's disease, the amount of intracellular accumulation of damaged tau protein and inter-synaptic spreading of the pathology positively correlates with the progression of AD and cognitive decline of patients (Alafuzoff et al. 2008). Spreading of tau pathology resembles infection behaviour of viruses or more closely prions or tauons as the transmitted particles were named more than 20 years ago (Novak 1994; Ball et al. 2013). The proteolytic processing and misfolding of amyloid precursor protein (APP) fragments does not correlate with disease progression so well, rather it potentiates and accelerates tau protein toxicity (Khan et al. 2014). In Parkinson's disease, parkin, leucine-rich repeat kinase 2 (LRRK2) and, more importantly, α-synuclein gradually accumulate and form presumably toxic proteinaceous fibrillary structures (Spillantini et al. 1997).

Whether or not are these high molecular protein aggregates the primary cause of the diseases or just a consequence or even protective in the process of neurodegeneration still remains a matter of debate. It is more likely that monomeric and/or oligomeric intermediates could play a more significant role at the onset of the diseases (Kovacech et al. 2009). Animal models show that transgenic expression of pathological form of human tau protein results in irreversible changes in brain and premature death of the experimental animals (Zilka et al. 2006; Filipcik et al. 2012). In PD, the accumulation of α-synuclein results in formation of Lewy bodies (Goedert et al. 2013).

The cellular regeneration capability of neurons depends exclusively on the function of the mechanisms controlling the quality of synthesis and degradation of proteins. Generally the lifespan of intracellular proteins, multicomponent complexes and organelles is variable and therefore the cells have to undergo periodic regenerative processes (Ciechanover and Brundin 2003). During this renewal process, the proteosynthetic machinery produces a certain amount of damaged or non-functional proteins which need to be removed. In tissues with a rapid cell turnover, the protein quality control is effectively accomplished by apoptosis, phagocytosis and cell division. In brain tissue, especially in neurons the cell regeneration is very limited, as there are only few areas in the adult brain where neurogenesis occurs, and therefore the cell division as a regeneration mechanism cannot be employed. The neurons in CNS use the UPS, autophago-lysosome and exocytosis in order to clear intracellular debris. Within the time, defective, misfolded and otherwise damaged proteins may become accumulated in long lived neurons, which is the process that can occur as a consequence of gradually lowering UPS activity during the ageing (Ciechanover and Kwon 2015).

Obviously, one of the key strategies in fighting neurodegeneration induced by the misfolded proteins and their toxic oligomers can therefore be the stimulation of their degradation. Concurrently with this process, the activity of selected heat shock proteins may result in increased level of substrates available for degradation in UPS. *Via* targeting this mechanism one could be able to prevent the initial conversion of the proteins into toxic molecules that accumulate in the intracellular space, elope into extracellular space, and progressively spread throughout the CNS, invading adjacent areas of the affected brain.

While the autophago-lysosome pathway is a mechanism responsible for the degradation of damaged or defective intracellular organelles and large protein aggregates, the ubiquitin-proteasome complex is responsible for precise and complete elimination of unusable, mutant, misfolded,

defective, terminally modified and accumulated proteins in both, cellular nucleus and cytoplasm (Ciechanover et al. 2000; Hartl and Hayer-Hartl 2009).

UPS, however, is not exclusively directed to protein degradation, it is also responsible for regulation of gene transcription by monoubiquitination and deubiqitination of histones, cell cycle regulation, apoptosis and in plants also to auxin-mediated response to light (Eide et al. 2005; Vissers et al. 2008). Furthermore, the role of UPS is indispensable in normal function of immune system (Brehm and Kruger 2015) and plays a role in highly specific control of protein expression in different signal-transduction pathways and intracellular communication throughout the regulation of synapses development in neurons (Leal et al. 2014).

UPS selective degrades proteins conjugated with ubiquitin, a small 8.5 kDa highly conserved protein (Hershko and Ciechanover 1998), composed of 76 amino acids that is present in every eukaryotic cell (Pickart and Eddins 2004). In mammals ubiquitin (Ub) is encoded by four different genes: *UBB*, *UBC*, *UBA52* and *UBA80* (Kimura and Tanaka 2010). Ubiquitination involves the covalent attachment of C terminal residue of ubiquitin *via* isopeptide bond to a lysine residue of target protein. This process includes cooperation of ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2) and finally ubiquitin ligase (E3). In the final step, ubiquitin monomers are activated by ATP-dependent reaction through the use of E1 and transferred to cysteine residues of E2. Subsequently, ubiquitin is attached to target protein by force of E3. E3 ligase is a link between target protein and E2-ubiquitin complex and mediates formation of covalent binding between C-terminal residues of ubiquitin monomer and lysine residues of target molecule. Another activated ubiquitin molecules are consequently added to first ubiquitin forming poly-ubiquitin chains (Chau et al. 1989; Gregori et al. 1990). Poly-ubiquitin chains can have different topography based on presence of seven lysine residues on ubiquitin itself: K6, K11, K27, K29, K33, K48 and K68 (Dennissen et al. 2012). Different chain architecture leads to different protein fate. For example, proteins bearing K48 linked ubiquitin-chain composed of four or more molecules are distinguished by 26S proteasome and subsequently degraded (Deveraux et al. 1994; Heride et al. 2014). The specificity to the whole process is given by selective binding of E2/E3 enzymes to the protein. Their activity is often regulated by phosphorylation. Ubiquitin monomers are released from proteins after targeting of proteins to the proteasome and recycled. Release of ubiquitin is under the control of deubiquitinating enzymes (DUBs), proteases which reverse isopeptide bond and thus control the status of protein ubiquitination and the level of mono-ubiquitin in cells (for review see Ristic et al. 2014). Within the multi-enzymatic steps which include E2 (about 40 different enzymes), the E3 ubiquitin ligases (more than 700 enzymes) and deubiquitylases (about 100 enzymes) that could be druggable the E3 ligases represent probably the most specific targets (Skaar et al. 2014).

Proteasome (26S proteasome) is composed of three subunits: 20S catalytic core and two 19S regulatory regions. 20S subunit has a cylindrical structure formed by four rings. Its structure encompasses six active proteolytic sites: two trypsin-like, two chymotrypsin-like and two peptidylglutamyl-like proteolytic activity sites (Rivett et al. 1995). 19S subunit is responsible for recognition and binding of polyubiquitinated proteins. Prior to degradation in 20S catalytic core of the proteasome the polyubiquitinated proteins are unfolded and deubiquitinated (Hershko and Ciechanover 1998). Interestingly, the 20S proteasome subunit can also exist in a free form. In this case, 20S is able to digest proteins directly in ATP-independent and ubiquitin-independent way (Sanchez-Lanzas and Castano, 2014).

Since the proteins like tau and α-synuclein are prone to aberrant modifications that are normally subject for UPS degradation it seems very likely that UPS can play an important role in early stages of neurodegenerative diseases. Therefore the elimination of these aberrant proteins *via* UPS in early stage of disease seems to be an attractive therapeutic strategy for treatment of the major neurodegenerative diseases.

Alzheimer's disease, tau protein and UPS

A significant research effort in the field of neurodegenerative diseases has been focused on explanation of the AD pathogenesis through the overproduction of β-amyloid, the cleavage product of APP, which in AD accumulates into senile plaques. However, decades of research in APP processing did not yield the expected outcome and drug development based solely on the amyloid hypothesis was not successful until now (Calcul et al. 2012). Understandably, the attention in the AD research is drawn towards protein tau, the second prominent protein in AD, as it was clearly shown that the distribution of pathological forms of tau positively correlates with the extent of neurodegeneration and degree of cognitive impairment in AD sufferers (Braak et al. 2006). Tau protein is now considered as one of the most promising targets for development of new generation of anti-AD strategies (Ballatore et al. 2012) with currently first clinical trial of active anti-Tau immunotherapy being tested in human (Kontsekova et al. 2014).

Via alternative splicing, six tau isoforms are expressed from *MAPT* gene, differing by presence or absence of one or two inserts (29 or 58 amino acids) in the N-terminus and by presence or absence of the second repeat in the microtubule-binding domain (MTBD) of the molecule (Goedert et al. 1989). Tau protein belongs to the group of phosphoproteins, its molecule contains more than 80 potential phosphorylation sites (Wang et al. 2013). Tau protein is predominantly localized in axons, where it participates on regulation of microtubule

stability and thus contributes to the determination of cell morphology. When phosphorylated at serine and threonine residues within MTBD the binding of tau to microtubule is diminished and it accumulates in somato-dendritic space of neuron (Kuret et al. 2005; Filipcik et al. 2009). Tau in AD patient brains is abnormally hyper-phosphorylated at multiple phosphorylation sites and pathologically truncated at both ends of the molecule (Novak et al. 1993; Iqbal et al. 2009; Lloret et al. 2015). Such pathologically modified tau proteins form paired helical filaments (PHF) that are the core constituents of neurofibrillary tangles (NFTs) (Novak et al. 1989).

Tau belongs to the group of intrinsically disordered proteins (IDP) that play an important role in many biological processes including cell signaling, translation, transcription regulation and facilitates multi-protein assemblies. Besides microtubules, tau protein was shown to interact with many other proteins, among which protein-phosphatases and heat-shock proteins (Hsp) are most prominent (Shimura et al. 2004; Dickey et al. 2007a). Under physiological conditions, the tau molecule remains in a highly soluble form. In fact, the loss of physiological tau function and gain of toxic properties is directly connected to the transformation of tau into an insoluble fibrillary structure – this seems to be common and most significant denominator of neurodegenerative tauopathies, including AD. The process of tau conversion into its pathological form includes at least two key transition steps. First is the posttranslational modification such as truncation followed by hyperphosphorylation, miss-phosphorylation, oxidation, nitration, glycation, glycosylation or others (Cente et al. 2009; Martin et al. 2011). The consequence of these changes is a decrease in microtubule-binding affinity and/or aberrant microtubule assembly. The second step is represented by the formation of hydrophobic bonds between two molecules of pathologically modified tau proteins in the cytoplasm, through the MTBD domains resulting in a beta sheet protein structure formation (Novak et al. 1993). Since the tau contains 3 or 4 MTBD the final PHF morphology depends on the specific tau isoform that PHF consist of (Sugino et al. 2009). It also depends on the presence of specific amino acid residues and presence of other molecules in the complex with tau. Aggregation of tau into various aberrant forms is a process that causes tau to gain its toxic properties (Cente et al. 2006; Gomez-Ramos et al. 2006; Nisbet et al. 2015). Inhibition or prevention of tau aggregation could be therefore an important step in the development of pharmacologic treatment of AD. Some scientific groups support the idea that the ideal drug candidate should inhibit tau hyperphosphorylation, since hyperphosphorylated tau aggregates much easier than its physiologically phosphorylated form, or directly inhibit the pathological conformation change that causes aggregation into PHF (Kosik and Shimura 2005; Iqbal et al. 2009; Ballatore et al. 2012). Together with tau miss-phosphorylation the tau cleavage leads to a conformational change and significantly increased susceptibility of truncated form to hyperphosphorylation and subsequent aggregation (Zilka et al. 2006). Obviously, the understanding of the causal mechanisms responsible for pathological tau cleavage is essential for further advancement in understanding of AD pathogenesis.

Tau protein and UPS

The major histopathological hallmark of neurofibrillary degeneration in Alzheimer's disease and related tauopathies is presence of tau protein deposits in the brain. Since the tau protein in these aggregates is highly posttranslationally modified it is still an open question how can be the tau degradation influenced by the posttranslational modifications. It was previously described that inhibition of proteasome in cell cultures causes inhibition of tau degradation (Tseng et al. 2008) and on the other hand exogenous delivery of purified proteasomes significantly promotes cell survival against proteotoxic stress caused by tau (Han et al. 2014). Furthermore, we have recently shown that pathologically truncated tau is degraded *via* proteasome pathway and simultaneously it is able to decrease the proteasome activity. Interestingly, in the same system, the inhibition of Hsp90 chaperone activity by geldanamycin increased the tau protein degradation and restored the proteasome activity, proving that enhanced clearance of pathological tau by UPS could represent promising strategy for treatment of neurodegeneration (Opattova et al. 2013). Other *in vitro* studies reported that abnormally truncated tau at D421 is predominantly degraded by autophagy, while full length tau protein is a subject for degradation by UPS (Dolan and Johnson 2010). Besides truncation, it was documented that tau hyperphosphorylation induced by okadaic acid almost completely blocked tau degradation in proteasome (Poppek et al. 2006). On the other hand the proteasome and calpain protease systems are capable of degrading tau in cell-free assays, albeit their inhibition does not alter cellular tau levels in primary neurons or differentiated neuroblastoma cells (Brown et al. 2005). It seems that selection of intracellular degradation pathway for tau protein depends not only on the specific posttranslational modifications but also on type of tau protein isoform. Although the degradation of tau protein in proteasome was intensively studied over the past few years the necessity of ubiquitination in the process is still discussed among the scientific community (Erales and Coffino 2014). Studies of various groups demonstrated that ubiquitination is not required for normal tau degradation (Grune et al. 2010) and tau as an intrinsically disordered protein can be degraded by the ubiquitin independent proteasome pathway, but it is also unclear how is the tau protein recognized by proteasome (Lee et al. 2013). On the contrary it is well known, that accumulated ubiquitin is present in the plaques and NFTs in the brains of AD patients (Kudo et al. 1994). PHF in AD brain have been reported as

monoubiquitinated (Morishima-Kawashima et al. 1993) and such proteins are poorly degraded by UPS. Importantly, it was shown that pathological structures in AD brains contain ubiquitin B mutated protein (UBB^{+1}), a mutated ubiquitin, which is created by deletion at its C-terminus (van Leeuwen et al. 1998). This protein is resistant to deubiquitination (Tan et al. 2007) and blocks the ubiquitin-dependent proteolysis in neural cells (Lindsten et al. 2002). Despite of increased level of ubiquitin in AD brains the E1 and E2 enzymes responsible for process of ubiquitination have been reported down-regulated in AD and this down-regulation is age-dependent (de Vrij et al. 2004). Some of the authors show that ubiquitination enhances tau aggregation, however, this has to be further investigated (Wang and Mandelkow 2012).

In addition to ubiquitination, total proteasome level and its activity plays an important role in AD pathogenesis. Brain areas affected by AD display noticeable decrease in proteasome immunoreactivity, both the number of proteasome subunits and overall proteasome activity are lowered (Keller et al. 2000; Lehman 2009). Moreover, insoluble tau aggregates isolated from human AD brains directly induce proteasome dysfunction resulting from the inhibitory binding of PHF-tau to the proteasome (Keck et al. 2003).

Ageing significantly contributes to the decrease of proteasome activity (Dasuri et al. 2009; Vilchez et al. 2014). Therefore, it is not surprising that in etiopathogenesis of aging-associated neurodegenerative disorders including AD and PD we can observe the markers of proteostasis failure. Dysregulation of individual processes of proteostasis manifested by decreased number of functional proteasomes together with inhibition of proteasome activity and impaired ubiquitination inevitably results to impairment of protein degradation. Inhibition of tau protein degradation can lead to generation of tau pro-aggregating forms capable to promote formation of tau oligomers (Wang et al. 2009). Considering recent data, the strategies targeting enhancement of degradation pathways could influence the generation of toxic tau fragments and reactivate the impaired proteostasis in AD (Fig. 1). Still it is

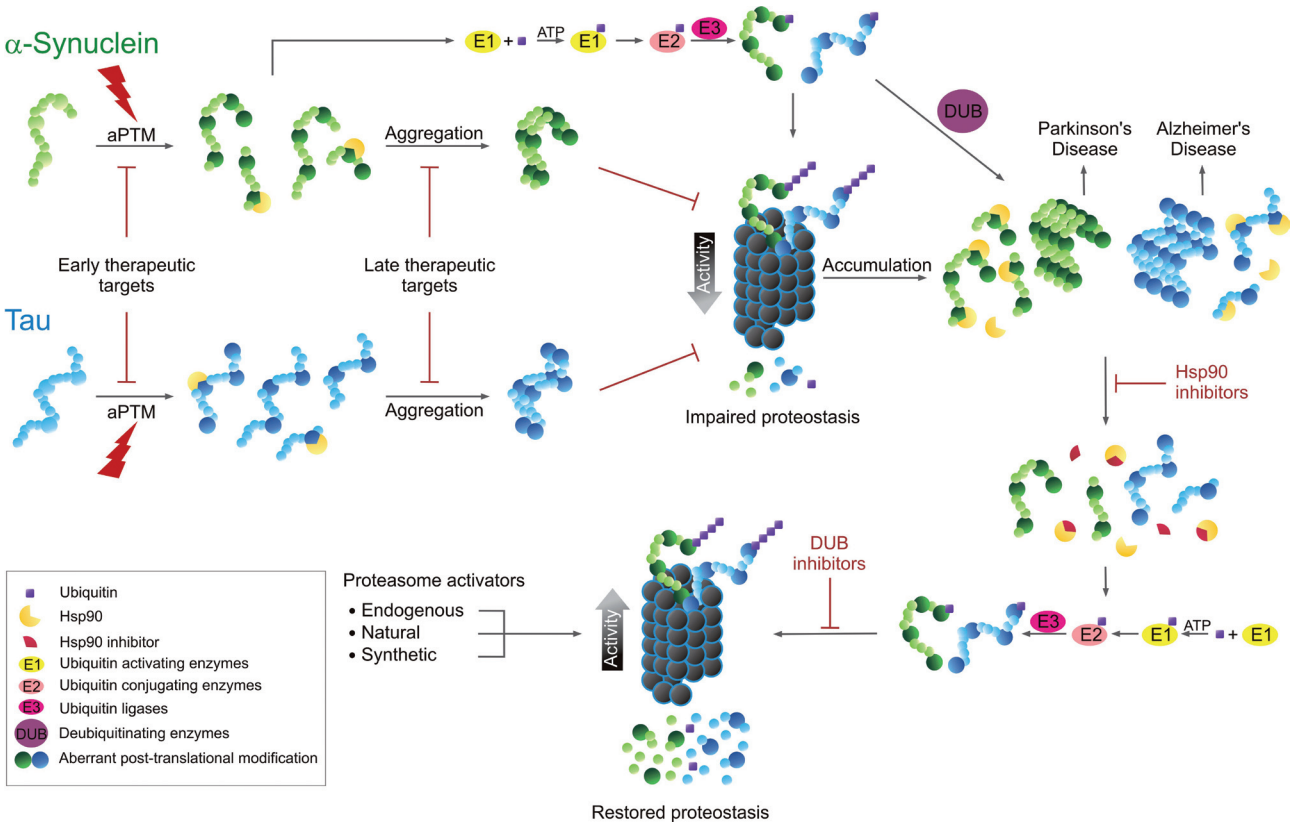

Figure 1. The pathophysiological mechanisms in Alzheimer's and Parkinson's diseases and potential therapeutic targets in ubiquitin proteasome system for their treatment. Abnormal posttranslational modifications of both α-synuclein and protein tau, such as truncation, hyperphosphorylation and others can lead to protein aggregation and generation of high molecular assemblies, which are characteristic pathognomic feature of the diseases. High molecular aggregates inhibit activity of proteasome, which accelerates further generation of the aggregates. In contrast, the inhibition of Hsp90 activity, inhibition of deubiquitinase and activation of proteasome are the processes that may alleviate neurodegeneration *via* acceleration of toxic protein clearance.

the open question to what extent this process could be AD specific and how to avoid the neurotoxicity, which is the major concern accompanied with this treatment strategy.

Parkinson's disease, α-synuclein and UPS

The most significant pathological sign of Parkinson's disease is a progressive degeneration of dopaminergic neurons in *substantia nigra*. Histopathological hallmarks of PD include the presence of Lewy neurites along with intraneuronal insoluble aggregates called Lewy bodies (LB), which are composed of a heterogenic mix of proteins and lipids (McNaught et al. 2003). However, the major protein component of Lewy bodies is α-synuclein (Spillantini et al. 1997). LBs are localized in the cytoplasm of dopaminergic neurons in the *substantia nigra* and neurons of cerebellar cortex and magnocellular basal ganglia (Braak et al. 1995). Pathological form of α-synuclein was shown in the brains of patients with sporadic forms of AD as well (Jellinger 2011). Aside from the classic motoric impairment in PD, synucleinopathy is also the neurodegenerative process behind dementia with Lewy bodies (DLB), which features cognitive impairment combined with hallucinations, brain atrophy, and accumulation of LB in cortical or cholinergic brain areas (nucleus basalis of Meynert) affected also in patients with AD (Liu et al. 2015). Definite pathogenic role of α-synuclein was confirmed by identification of point mutations and duplications/triplications in α-synuclein gene (SNCA locus), which were shown to be causative for familial forms of PD (Hardy et al. 2009; Deng and Yuan 2014). α-synuclein present in LB is phosphorylated predominantly at serine 129 (pS129) and can be also oxidised (Spillantini et al. 1997) or alternatively modified, for example proteolytically cleaved (Breydo et al. 2012). The degradation of α-synuclein takes place in ubiquitin-proteasome system (Bennett et al. 1999), however, it can be degraded also by autophagy (Mak et al. 2010) and by chaperone-mediated autophagy (Cuervo et al. 2004). The failure in elimination of α-synuclein aggregates from the neurons is indicative of defective proteostasis.

α-synuclein and UPS

Significant role of UPS in the PD pathology is supported by discovery of ubiquitin in Lewy bodies (Beyer et al. 2009). The ability of UPS to recognize misfolded proteins as well as labelling of misfolded proteins with ubiquitin and their consequent intracellular accumulation point to the fact, that the critical factor in the clearance is a degradation step rather than ubiquitination. This means that pathological proteins could be marked for degradation, however, the degradation process fails, or is not complete and may moreover generate still more toxic fragments. It was shown that experimentally-induced overexpression of wild type α-synuclein, as well as its mutated form inhibits proteasome activity (Nonaka and Hasegawa 2009; Emmanouilidou et al. 2010). The UPS inhibition in PC12 cells leads to the increase in accumulation of ubiquitin positive α-synuclein aggregates, which further indicates the significance of a deficient proteasome processing system in PD pathology (Rideout et al. 2001; Martinez-Vicente and Vila 2013). It was also shown that aggregated α-synuclein directly interacts with the 19S proteasome subunit, resulting in inhibition of the protein interactions with the catalytic subunit, and finally in the blocking of the 26S proteasome subunit (Snyder et al. 2003; Emmanouilidou et al. 2010). Defects in UPS function were documented not only in cellular and animal PD models, but in sporadic PD patients as well. In the brains of PD patients, we can observe a selective decrease in proteasome activity and reduction of proteasome subunits in the *substantia nigra*, but not in the striatum or cortex (McNaught et al. 2003). This indicates that UPS deficiency contributes to dopaminergic neurodegeneration in *substantia nigra* in sporadic PD patients (Betarbet et al. 2005). The connection between defects in UPS and development of PD neurodegeneration is also supported by familial forms of PD. Mutations in parkin gene as well as in the „ubiquitin carboxy-terminal hydrolase L1"(*UCHL1*) genes are directly linked to UPS activity. Parkin, which is an ubiquitin/ligase (E3) cooperates with ubiquitin-conjugation enzymes UbcH7 and UbcH8 (E2). Mutations related to familial form of PD inhibit binding between parkin and E2 enzymes and consequently cause the loss of E3 ligase function. Mutations can also decrease parkin enzymatic activity and interaction with C-terminus of Hsc70 Interacting Protein, CHIP (Imai et al. 2002). These interactions result in defects of substrate ubiquitination and transfer to UPS. Interestingly, parkin over-production prevents proteasome defects caused by α-synuclein (Petrucelli et al. 2002). Parkin also influences ubiquitination of the mitochondrial proteome, which significantly affects PD pathogenesis (Sarraf et al. 2013). UCHL1 belongs to the group of de-ubiquitination enzymes and is responsible for hydrolysis of poly-ubiquitinated chains. Mutations in the *UCHL1* gene decrease its catalytic activity, which in PD leads to decrease of ubiquitination and finally to the failure of misfolded protein degradation (Gong and Leznik 2007). The above mentioned experimental evidence strongly supports the significance of proteasome activity in the etiopathogenesis of PD.

Activation of proteasome: a promising treatment strategy?

Ubiquitin proteasome system belongs to the major molecular machinery focused to keep cells in physiological conditions; it is indispensable for cell survival and therefore its regulation should be very careful since the side effects of the regulators could be life threatening. Still the activation of proteasome

pathways is considered as one of the potential therapeutic strategies for treatment of AD and PD (Dantuma and Bott 2014). There are 3 principal ways of the UPS activation: endogenous activators, genetic activators and natural or synthetic compounds, small molecules.

To date, three main endogenous 20S proteasome activators were described: PA28, PA200 and PA700 (Schmidt and Finley 2014). PA28 activates peptide hydrolysis by association of 20S proteasome with it α rings (Rechsteiner et al. 2000). Similarly to PA200, this pathway is ATP and ubiquitin-independent and is not involved in degradation of ubiquitinated proteins. PA28 is also known as 11S regulatory particle (REG). This regulatory particle is often a hetero-hepta-dimer localized in cytoplasm or homo-hepta-dimer localized mainly in the nucleus (Mao et al. 2008). Overexpression of PA28 was shown to enhance the survival of neurons in Huntington disease cell model (Seo et al. 2007). This suggests that proteasome activators may be used as therapeutic targets (Huang and Chen 2009). PA200 is 200 kDa nuclear protein, which binds to 20S proteasome (Ustrell et al. 2002). This proteasome activator enhances hydrolysis of peptides, most notably after acidic residues (Blickwedehl et al. 2008). PA700-19S regulatory particle is 1 MDa complex which consists of 19 proteins. This regulatory particle activates 20S proteasomes in an ubiquitin- and ATP-dependent manner. The individual subunits of this particle are involved in de-ubiquitination, unfolding of proteins and α-ring gate opening and facilitate substrate entry to the proteasome. PA28-20S-PA700 also forms the hybrid proteasomes, which are involved with 26S proteasome in MHCI antigen presentation (Hendil et al. 1998). Induction of PA700 has been considered as one of the possible therapeutic strategies *via* degradation of poly-ubiquitinated proteins.

The group of genetic activators is currently represented by only one yeast transcriptional regulatory system of proteasome, a zinc finger transcription factor Rpn4, which is a proteasome-associated transcriptional regulator carrying proteasome-associated control elements (PACE) sequence (Karpov et al. 2008). Studies on human fibroblast cellular model indicate that up-regulation of proteasome activity may be achieved also by overexpression of the proteasome maturation protein (POMP), which elevates the level of functional and assembled proteasomes and enhances the antioxidant capacity of cells (Chondrogianni and Gonos 2007).

Over the period of time several small molecules able to activate proteasome were identified. This group of molecules includes denaturing reagents, lipids, peptides, fatty acids, synthetic peptidyl alcohols, esters and nitriles activators, such as SDS, polylysine and linoleic acid. The mechanism of action of SDS and fatty acids consists in partial denaturation of proteasome and opening of its conformation (Watanabe and Yamada 1996). Synthetic peptidyl alcohols, esters, p-nitroanilines and nitriles activate proteasome through PA28 binding site (Wilk and Chen 1997). Potent proteasome activators are also betulinic acid and oleuropein, isolated from *Betula pubescens* and *Olea europaea*, respectively. Betulinic acid dominantly activates chymotrypsin-like proteasome activity, while oleuropein can activate all three proteasomal activities and cause conformational changes of the 20S α-ring (Katsiki et al. 2007). Other natural antioxidants, such as dithiolethione and sulforaphane may increase expression of proteasome and enhance protection against oxidative stress (Kwak et al. 2007). From the relatively large spectrum of proteasome activators only few of them were tested for their efficacy in neurodegenerative processes. Those which were found effective in experimental models of neurodegenerative disease are summarized in Table 1.

Another strategy for enhancement of protein degradation is stimulation of ubiquitination. This approach of selective ubiquitination and proteasome post-translational degradation of specific substrates employs proteolysis targeting chimera molecules (PROTACS) (Prabha et al. 2012). PROTACS are interesting, hetero-bi-functional molecules, which act as a bridge between targeted protein and E3 ligase. E3 ubiquitin

Table 1. Efficacy of proteasome activators in experimental models of neurodegenerative diseases

Compound	Mechanism of action	Effect	Experimental model	References
Betulinic acid	Activation of chymotrypsin proteasome activity	Enhanced clearance of tau and amyloid β	MT4 human T cells	Friedman et al. 2014; Huang et al. 2007
Oleuropein	Activation of trypsin, chymotrypsin and caspase activity of proteasome	Inhibition of tau aggregation	*In vitro* aggregation assay with P301L tau protein	Daccache et al. 2011
Dithiolethione	Increase in proteasomal peptidase activities	Neuroprotection	Hydroxydopamine treated SHSY5Y cells	Brown et al. 2014
Sulforaphane	Induction of proteasome activities	Protection against Ab4 induced neurotoxicity	Scopolamine treated mice/ Neuro2A	Lee et al. 2014; Park et al. 2009
Lithocholic acid derivatives	Activation of chymotrypsin proteasome activity	Alleviation of Aβ induced inhibition of proteasome	*In vitro* assay with amyloid $\beta_{1\text{-}42}$	Dang et al. 2012

ligase with bound PROTAC promotes synthesis of polyubiquitin chain on the target protein which leads to facilitation of recognition and subsequent degradation of target proteins by 26S proteasome (Cyrus et al. 2011).

Heat shock proteins in neurodegeneration

Regulation of proteostasis *via* heat shock proteins appears to be very interesting strategy for suppression of neurodegeneration. Hsps are chaperones that significantly influence protein quality control and are responsible for correct structural assembly of nascent polypeptide chains and preservation its structure during the transportation to the effector site of the protein. Those proteins with defective structure that can't be reassembled by chaperones are ubiquitinated and consequently degraded in proteasome (Goryunov and Liem 2007). Different chaperones use different effector mechanisms. Their expression is induced predominantly as a response mechanism to various external factors including heat stress, hypoxia and presence of heavy metals or amino acids analogues (Chen et al. 2007). Mammalian cells contain more than 100 proteins of the chaperone family that are classified into groups according to their molecular weight. The most significant among them are Hsp110, Hsp90, Hsp70, Hsp60, Hsp40 a Hsp27. Immunological and biochemical studies have shown increased expression of Hsp proteins in AD brains (Sahara et al. 2005). Recently, Hsp27, Hsp70 and CHIP were described to have the ability to recognize abnormal tau and reduce its concentration by mediating its degradation and dephosphorylation. Hsp27 was described as a preferential binding partner of hyperphosphorylated tau (Shimura et al. 2004). The amount of proteins, especially Hsp27, Hsp40, Hsp90, alfaB-crystaline and CHIP positively correlates with the amount of soluble tau protein (Sahara et al. 2007), and inverse correlation with the level of granulated tau oligomers, the intermediates of NFT formation was described (Koren et al. 2009). However, the communication between Hsp27 and pathological tau protein is indirect and more complicated than previously thought (Filipcik et al. 2015).

Postmortem analysis of PD brains showed that Hsp90, Hsc70 and Hsp40 co-localize with α-synuclein in Lewy bodies (Uryu et al. 2006).

Functioning of the chaperones is generally positive and leads to recovery of proteostasis and establishment of physiological status, however, there is at least one interesting example clearly showing how the originally physiological function of chaperone can be transformed into neuropathogenic. Hsp90 normally stabilizes the proteins, securing their correct structural assembly (Wandinger et al. 2008). It contains two binding domains: C- and N-terminal (Garnier et al. 2002). The C-terminal region modulates its N-terminal ATPase activity (Owen et al. 2002). It is known that Hsp90 binds to GSK3β and inhibits tau protein phosphorylation (Dickey et al. 2007a). Recent studies, however, revealed that pathologically modified tau protein induces Hsp90 to act as a stabilizer of such a pathologic conformational change of tau

Table 2. Efficacy of Hsp90 inhibitors in experimental models of neurodegenerative diseases

Compound	Hsp90 binding	Family	BBB permeability	Effect	Experimental model	References
17AAG	N-term	Hydroquinone	Good	Increases clearance of tau	P301L tau mouse β amyloid model (Tg2576)	Ho et al. 2013
17DMAG	N-term	Hydroquinone	Good	Increases clearance of tau	Rat primary corticohippocampal neurons expressing $T_{151\text{-}391}$	Opattova, unpublished data
PU-H71	N-term	Purine	Poor	Inhibits of LRRK2-toxicity and rescued axon growth defect	Drosophila ortholog (CG5483) of human LRRK1 and LRRK2	Wang et al. 2008
EC102	N-term	Purine	Good	Decreases phosphorylated tau	P301L cellular model	Dickey et al. 2006
PU24FCL	N-term	Purine	Good	Increases Hsp70	Primary neurons	Luo et al. 2007
SNX-9114	N-term	Pyrazole	Good	Protects against α-synuclein induced loss of dopaminergic neurons	Rat model of parkinsonism (α-synuclein)	McFarland et al. 2014
KU-32	C-term	Novobiocin derivate	Good	Protects against Aβ-induced toxicity	SHSY5Y cell cultures	Lu et al. 2009

BBB, blood brain barrier; 17AAG, 17-allylamino-17-demethoxygeldanamycin; 17DMAG, 17-dimethylaminoethylamino-17-demethoxygeldanamycin; PU-H71, 6-Amino-8-[(6-iodo-1,3-benzodioxol-5-yl)thio]-*N*-(1-; methylethyl)-9*H*-purine-9-propanamine; EC102, C19H25IN6OS; PU24FCL, 8-aryl-sulfanyl adenine; SNX-9114, derivative of 4-[6,6-dimethyl-4-oxo-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazol-1-yl]-2-[(trans-4-hydroxycyclohexyl)amino]-benzamid; KU-32, [N-(7-((2R,3R,4S,5R)-3,4-dihydroxy-5-methoxy-6,6-dimethyl-tetrahydro-2H-pyran-2-yloxy)-8-methyl-2-oxo-2H-chromen-3-yl)acetamide].

that is vulnerable to hyperphosphorylation and aggregation and consequently increases intraneuronal concentration of pathological tau protein intermediates (Tortosa et al. 2009). Not surprisingly, the increased Hsp90 chaperone activity than leads to the progression of the neurodegenerative phenotype (Blair et al. 2013). Logical consequence was testing of Hsp90 activity inhibitors for their potential therapeutic effect in models of neurodegenerative disorders. This led to development of several candidate molecules for treatment of AD and PD. The list of the molecules used in this effort and their basic features are included in Table 2.

Inhibitors of Hsp: stimulators of proteasome?

In contrast to the current treatment procedures established for AD and PD that help to manage the symptoms of the disease, the regulation of UPS could be a promising candidate for causal therapy. Targeting the formation of the initial oligomers that gain the toxic function and initiate the neurodegenerative process should stop the disease at the very beginning. Particularly Hsp90, which is a promising target for treatment of cancer, seems to be an important target for therapy of neurodegeneration as well (Gallo 2006; Shimamura and Shapiro 2008). Hsp90 is a prominent heat shock protein, which composes about 1–2% of all cytosolic proteins (Pratt and Toft 2003). Hsp90 interacts with more than 20 partner proteins, most of which are co-chaperones that regulate its activity (Li et al. 2012). It plays an important role in the structural assembly of unfolded proteins (Chiosis et al. 2006) and in protein maturation. Non-toxic inhibition of Hsp90 in AD models activates the heat shock factor 1 (HSF1), a transcription factor, which controls the production of Hsp70, Hsp40 and Hsp27, the proteins taking place in the process of elimination of tau protein aggregates (Luo et al. 2007). Induction of Hsp70, Hsp40 and Hsp27 expression leads to selective elimination of phosphorylated and conformationally transformed tau proteins by proteasome pathway, which in conclusion prevents the toxicity associated with pathological tau (Dickey et al. 2007b; Opattova et al. 2013). The first identified Hsp90 inhibitor was geldanamycin (GDA), a natural product extracted from bacteria *Streptomyces hygroscopicus*. It belongs to the group of benzochinone ansamycine antibiotics and binds Hsp90 through its N-terminal domain (Hartson et al. 1999). The inhibition of Hsp90 activity by GDA increases expression of Hsp90 and Hsp70, which results in a decrease in the level of hyperphosphorylated aggregated tau (Petrucelli et al. 2004); moreover it also reduces extracellular α-synuclein oligomers formation and related toxicity (Danzer et al. 2011). GDA inhibits the function of Hsp90 by reduction of its ATPase activity (Panaretou et al. 1998), which results in the increase of client protein degradation and the further HSF1 activation (Ciechanover and Brundin, 2003). Other natural Hsp90 inhibitors are herbimycin, radicicol, novobiocin and its analogs, coumermycin A1, taxol and other (Amolins and Blagg 2009). Synthetic analogues of GDA such as 17-AAG a 17-DMAG (17-(dimetylaminoetilamino)-17-demetoxy-geldanamycine) are characterized by a lower cytotoxicity and higher solubility than GDA (Jez et al. 2003; Ortega et al. 2014). It has been observed that inhibitor of Hsp90 increases the activity of proteasome (Pajonk and McBride 2001; Opattova et al. 2013); although the mechanism of this activity is not yet clear, this would be an ideal combination of effects induced by one molecule. Relatively large amount of Hsp90 inhibitors was synthetized to date (Blair et al. 2013). Although for treatment of cancer several of them entered clinical testing and seems to be promising for special forms of neoplasia (Neckers and Workman 2012; Jhaveri et al. 2014), till now no inhibitor of Hsp90 that would have ideal properties for clinical use in AD or PD patients was developed. Therefore an effort should be done in order to develop the inhibitors with acceptable pharmacokinetic properties, good bioavailability including good blood brain barrier transfer. It is likely that non-toxic inhibitors of Hsp90 could lead to re-establishment of the proteostasis; however, they should always be tested for the prevention of cognitive decline and behavioural deficits, the two phenomena, which are typical for AD and PD (Fig. 1).

Conclusion

Neurodegenerative disorders lead to a decrease of cognitive abilities, memory impairment and behavioural deficits in a significant part of the elderly population. Alzheimer's and Parkinson's diseases, despite a difference in symptomatology, share common signs at the subcellular and molecular level, the most significant is an accumulation of misfolded proteins which gain toxic functions and drive the neuropathology of these fatal diseases. Considering the common denominator in molecular mechanism, the concept of treatment possibilities could be similar in both of these disorders. Since the chaperone system, originally focused on the repair of the defective proteins, can also prolong the lifespan of toxic proteins in neurons, the inhibition of this system could lead to increased degradation of toxic proteins, which may result in improvement of neuron survival and consequently to the alleviation of neurodegeneration. Concurrently, specific activation of degradation signaling pathways e.g. ubiquitin proteasome system could be an attractive tool in fighting against neurodegeneration. The combination of these two approaches could lead to significant decrease of amount of intraneuronal misfolded proteins or completely eliminate toxic proteins from neurons. Major bottleneck in this

strategy is the lack of compounds that would specifically target just pathologically modified proteins and leave the physiological ones untouched. Such a complex system as UPS provides good opportunity for development of specific therapeutics targeting specific groups of misfolded proteins, the amount of potential candidates is huge. Another biggest obstacle, which remains to be solved, is the toxicity and adverse effects of the compounds targeting UPS or chaperone system. The idea of re-establishment of neuroproteostasis is appealing; the way from this idea to the experimental evidence at least in preclinical animal models will be still very long and tough. Once it comes to the practice, very likely this strategy could be efficient not only in AD and PD, but also in other neurodegenerative diseases with related molecular pathology, which could be a major step on the route to the healthy ageing.

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References

Alafuzoff I., Arzberger T., Al-Sarraj S., Bodi I., Bogdanovic N., Braak H., Bugiani O., Del-Tredici K., Ferrer I., Gelpi E. et al. (2008): Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. Brain Pathol. **18,** 484–496 http://dx.doi.org/10.1111/j.1750-3639.2008.00147.x

Amolins M. W., Blagg B. S. (2009): Natural product inhibitors of Hsp90: potential leads for drug discovery. Mini Rev. Med. Chem. **9,** 140–152 http://dx.doi.org/10.2174/138955709787316056

Ball M. J., Lukiw W. J., Kammerman E. M., Hill J. M. (2013). Intracerebral propagation of Alzheimer's disease: strengthening evidence of a herpes simplex virus etiology. Alzheimers. Dement. **9,** 169–175 http://dx.doi.org/10.1016/j.jalz.2012.07.005

Ballatore C., Crowe A., Piscitelli F., James M., Lou K., Rossidivito G., Yao Y., Trojanowski J. Q., Lee V. M., Brunden K. R., Smith A. B. 3rd. (2012): Aminothienopyridazine inhibitors of tau aggregation: evaluation of structure-activity relationship leads to selection of candidates with desirable in vivo properties. Bioorg. Med. Chem. **20,** 4451–4461 http://dx.doi.org/10.1016/j.bmc.2012.05.027

Ballatore C., Lee V. M., Trojanowski J. Q. (2007): Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat. Rev. Neurosci. **8,** 663–672 http://dx.doi.org/10.1038/nrn2194

Bedford L., Hay D., Paine S., Rezvani N., Mee M., Lowe J., Mayer R. J. (2008): Is malfunction of the ubiquitin proteasome system the primary cause of alpha-synucleinopathies and other chronic human neurodegenerative disease? Biochim. Biophys. Acta **1782,** 683–690 http://dx.doi.org/10.1016/j.bbadis.2008.10.009

Bennett M. C., Bishop J. F., Leng Y., Chock P. B., Chase T. N., Mouradian M. M. (1999): Degradation of alpha-synuclein by proteasome. J. Biol. Chem. **274,** 33855–33858 http://dx.doi.org/10.1074/jbc.274.48.33855

Betarbet R., Sherer T. B., Greenamyre J. T. (2005): Ubiquitin-proteasome system and Parkinson's diseases. Exp. Neurol. **191** (Suppl. 1), S17–27 http://dx.doi.org/10.1016/j.expneurol.2004.08.021

Beyer K., Domingo-Sabat M., Ariza A. (2009): Molecular pathology of Lewy body diseases. Int. J. Mol Sci. **10,** 724–745 http://dx.doi.org/10.3390/ijms10030724

Blair L. J., Nordhues B. A., Hill S. E., Scaglione K. M., O'Leary J. C. 3rd, Fontaine S. N., Breydo L., Zhang B., Li P., Wang L. et al. (2013): Accelerated neurodegeneration through chaperone-mediated oligomerization of tau. J. Clin. Invest. **123,** 4158–4169 http://dx.doi.org/10.1172/JCI69003

Blickwedehl J., Agarwal M., Seong C., Pandita R. K., Melendy T., Sung P., Pandita T. K., Bangia N. (2008): Role for proteasome activator PA200 and postglutamyl proteasome activity in genomic stability. Proc. Nat. Acad. Sci. USA **105,** 16165–16170 http://dx.doi.org/10.1073/pnas.0803145105

Braak H., Braak E., Yilmazer D., Schultz C., de Vos R. A., Jansen E. N. (1995): Nigral and extranigral pathology in Parkinson's disease. J. Neural Transm. Suppl. **46,** 15–31

Braak H., Alafuzoff I., Arzberger T., Kretzschmar H., Del Tredici K. (2006): Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. **112,** 389–404 http://dx.doi.org/10.1007/s00401-006-0127-z

Brehm A., Kruger E. (2015): Dysfunction in protein clearance by the proteasome: impact on autoinflammatory diseases. Semin. Immunopathol. DOI 10.1007/s00281-015-0486-4 http://dx.doi.org/10.1007/s00281-015-0486-4

Breydo L., Wu J. W., Uversky V. N. (2012): Alpha-synuclein misfolding and Parkinson's disease. Biochim. Biophys. Acta **1822,** 261–285 http://dx.doi.org/10.1016/j.bbadis.2011.10.002

Brown D. A., Betharia S., Yen J. H., Tran Q., Mistry H., Smith K. (2014): Synthesis and structure-activity relationships study of dithiolethiones as inducers of glutathione in the SH-SY5Y neuroblastoma cell line. Bioorg. Med. Chem. Lett. **24,** 5829–5831 http://dx.doi.org/10.1016/j.bmcl.2014.10.005

Brown M. R., Bondada V., Keller J. N., Thorpe J., Geddes J.W. (2005): Proteasome or calpain inhibition does not alter cellular tau levels in neuroblastoma cells or primary neurons. J. Alzheimers Dis. **7,** 15–24

Calcul L., Zhang B., Jinwal U. K., Dickey C. A., Baker B. J. (2012): Natural products as a rich source of tau-targeting drugs for Alzheimer's disease. Future Med. Chem. **4,** 1751–1761 http://dx.doi.org/10.4155/fmc.12.124

Cente M., Filipcik P., Pevalova M., Novak M. (2006): Expression of a truncated tau protein induces oxidative stress in a rodent model of tauopathy. Eur. J. Neurosci. **24,** 1085–1090 http://dx.doi.org/10.1111/j.1460-9568.2006.04986.x

Cente M., Mandakova S., Filipcik P. (2009): Memantine prevents sensitivity to excitotoxic cell death of rat cortical neurons expressing human truncated tau protein. Cell. Mol. Neurobiol. **29,** 945–949

http://dx.doi.org/10.1007/s10571-009-9379-1
Chau V., Tobias J. W., Bachmair A., Marriott D., Ecker D. J., Gonda D. K., Varshavsky A. (1989): A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. Science **243,** 1576–1583
http://dx.doi.org/10.1126/science.2538923
Chen Y., Voegeli T. S., Liu P. P., Noble E. G., Currie R.W. (2007): Heat shock paradox and a new role of heat shock proteins and their receptors as anti-inflammation targets. Inflamm. Allergy Drug Targets **6,** 91–100
http://dx.doi.org/10.2174/187152807780832274
Chiosis G., Caldas Lopes E., Solit D. (2006): Heat shock protein-90 inhibitors: a chronicle from geldanamycin to today's agents. Curr. Opin. Investig. Drugs **7,** 534–541
Chondrogianni N., Gonos E. S. (2007): Overexpression of hUMP1/POMP proteasome accessory protein enhances proteasome-mediated antioxidant defence. Exp. Gerontol. **42,** 899–903
http://dx.doi.org/10.1016/j.exger.2007.01.012
Ciechanover A., Orian A., Schwartz A. L. (2000): The ubiquitin-mediated proteolytic pathway: mode of action and clinical implications. J. Cell. Biochem. Suppl. **34,** 40–51
http://dx.doi.org/10.1002/(SICI)1097-4644(2000)77:34+<40::AID-JCB9>3.0.CO;2-6
Ciechanover A., Brundin P. (2003): The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. Neuron **40,** 427–446
http://dx.doi.org/10.1016/S0896-6273(03)00606-8
Ciechanover A., Kwon Y. T. (2015): Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. Exp. Mol. Med. **47,** e147
http://dx.doi.org/10.1038/emm.2014.117
Cuervo A. M., Stefanis L., Fredenburg R., Lansbury P. T., Sulzer D. (2004): Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science **305,** 1292–1295
http://dx.doi.org/10.1126/science.1101738
Cyrus K., Wehenkel M., Choi E. Y., Han H. J., Lee H., Swanson H., Kim K. B. (2011): Impact of linker length on the activity of PROTACs. Mol. Biosyst. **7,** 359–364
http://dx.doi.org/10.1039/C0MB00074D
Daccache A., Lion C., Sibille N., Gerard M., Slomianny C., Lippens G., Cotelle P. (2011): Oleuropein and derivatives from olives as Tau aggregation inhibitors. Neurochem. Int. **58,** 700–707
http://dx.doi.org/10.1016/j.neuint.2011.02.010
Dang Z., Jung K., Qian K., Lee K. H., Huang L., Chen C. H. (2012): Synthesis of lithocholic acid derivatives as proteasome regulators. ACS Med. Chem. Lett. **3,** 925–930
http://dx.doi.org/10.1021/ml3001962
Dantuma N. P., Bott L. C. (2014): The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. Front. Mol. Neurosci. **7,** 70
http://dx.doi.org/10.3389/fnmol.2014.00070
Danzer K. M., Ruf W. P., Putcha P., Joyner D., Hashimoto T., Glabe C., Hyman B. T., McLean P. J. (2011): Heat-shock protein 70 modulates toxic extracellular alpha-synuclein oligomers and rescues trans-synaptic toxicity. FASEB J. **25,** 326–336
http://dx.doi.org/10.1096/fj.10-164624
Dasuri K., Zhang L., Ebenezer P., Liu Y., Fernandez-Kim S. O., Keller J. N. (2009): Aging and dietary restriction alter proteasome biogenesis and composition in the brain and liver. Mech. Ageing Dev. **130,** 777–783
http://dx.doi.org/10.1016/j.mad.2009.10.003
de Vrij F. M., Fischer D. F., van Leeuwen F. W., Hol E. M. (2004): Protein quality control in Alzheimer's disease by the ubiquitin proteasome system. Prog. Neurobiol. **74,** 249–270
http://dx.doi.org/10.1016/j.pneurobio.2004.10.001
Deng H., Yuan L. (2014): Genetic variants and animal models in SNCA and Parkinson disease. Ageing Res. Rev. **15,** 161–176
http://dx.doi.org/10.1016/j.arr.2014.04.002
Dennissen F. J., Kholod N., van Leeuwen F. W. (2012): The ubiquitin proteasome system in neurodegenerative diseases: culprit, accomplice or victim? Prog. Neurobiol. **96,** 190–207
http://dx.doi.org/10.1016/j.pneurobio.2012.01.003
Deveraux Q., Ustrell V., Pickart C., Rechsteiner M. (1994): A 26 S protease subunit that binds ubiquitin conjugates. J. Biol. Chem. **269,** 7059–7061
Dickey C. A., Kamal A., Lundgren K., Klosak N., Bailey R. M., Dunmore J., Ash P., Shoraka S., Zlatkovic J., Eckman C. B. et al. (2007a): The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J. Clin. Invest. **117,** 648–658
http://dx.doi.org/10.1172/JCI29715
Dickey C. A., Patterson C., Dickson D., Petrucelli L. (2007b): Brain CHIP: removing the culprits in neurodegenerative disease. Trends Mol. Med. **13,** 32–38
http://dx.doi.org/10.1016/j.molmed.2006.11.003
Dickey C. A., Yue M., Lin W. L., Dickson D. W., Dunmore J. H., Lee W. C., Zehr C., West G., Cao S., Clark A. M. et al. (2006): Deletion of the ubiquitin ligase CHIP leads to the accumulation, but not the aggregation, of both endogenous phospho- and caspase-3-cleaved tau species. J. Neurosci. **26,** 6985–6996
http://dx.doi.org/10.1523/JNEUROSCI.0746-06.2006
Dolan P. J., Johnson G. V. (2010): A caspase cleaved form of tau is preferentially degraded through the autophagy pathway. J. Biol. Chem. **285,** 21978–21987
http://dx.doi.org/10.1074/jbc.M110.110940
Eide E. J., Woolf M. F., Kang H., Woolf P., Hurst W., Camacho F., Vielhaber E. L., Giovanni A., Virshup D. M. (2005): Control of mammalian circadian rhythm by CKIepsilon-regulated proteasome-mediated PER2 degradation. Mol. Cell. Biol. **25,** 2795–2807
http://dx.doi.org/10.1128/MCB.25.7.2795-2807.2005
Emmanouilidou E., Stefanis L., Vekrellis K. (2010): Cell-produced alpha-synuclein oligomers are targeted to, and impair, the 26S proteasome. Neurobiol. Aging **31,** 953–968
http://dx.doi.org/10.1016/j.neurobiolaging.2008.07.008
Erales J., Coffino P. (2014): Ubiquitin-independent proteasomal degradation. Biochim. Biophys. Acta **1843,** 216–221
http://dx.doi.org/10.1016/j.bbamcr.2013.05.008
Filipcik P., Cente M., Krajciova G., Vanicky I., Novak M. (2009): Cortical and hippocampal neurons from truncated tau transgenic rat express multiple markers of neurodegeneration. Cell. Mol. Neurobiol. **29,** 895–900
http://dx.doi.org/10.1007/s10571-009-9372-8
Filipcik P., Cente M., Zilka N., Smolek T., Hanes J., Kucerak J., Opattova A., Kovacech B., Novak M. (2015): Intraneuronal accumulation of misfolded tau protein induces overexpres-

sion of Hsp27 in activated astrocytes. Biochim. Biophys. Acta **1852,** 1219–1229
http://dx.doi.org/10.1016/j.bbadis.2015.03.003

Filipcik P., Zilka N., Bugos O., Kucerak J., Koson P., Novak P., Novak M. (2012): First transgenic rat model developing progressive cortical neurofibrillary tangles. Neurobiol. Aging **33,** 1448–1456
http://dx.doi.org/10.1016/j.neurobiolaging.2010.10.015

Friedman L. G., Price K., Lane R. F., Carman A. J., Dacks P. A., Shineman D. W., Fillit H. M. (2014): Meeting report on the Alzheimer's Drug Discovery Foundation 14th International Conference on Alzheimer's Drug Discovery. Alzheimers Res. Ther. **6,** 22
http://dx.doi.org/10.1186/alzrt252

Gallo K. A. (2006): Targeting HSP90 to halt neurodegeneration. Chem. Biol. **13,** 115–116
http://dx.doi.org/10.1016/j.chembiol.2006.02.003

Garnier C., Lafitte D., Tsvetkov P. O., Barbier P., Leclerc-Devin J., Millot J. M., Briand C., Makarov A. A., Catelli M. G., Peyrot V. (2002): Binding of ATP to heat shock protein 90: evidence for an ATP-binding site in the C-terminal domain. J. Biol. Chem. **277,** 12208–12214
http://dx.doi.org/10.1074/jbc.M111874200

Goedert M., Spillantini M. G., Del Tredici K., Braak H. (2013): 100 years of Lewy pathology. Nat. Rev. Neurol. **9,** 13–24
http://dx.doi.org/10.1038/nrneurol.2012.242

Goedert M., Spillantini M. G., Jakes R., Rutherford D., Crowther R. A. (1989): Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron **3,** 519–526
http://dx.doi.org/10.1016/0896-6273(89)90210-9

Golde T. E., Borchelt D. R., Giasson B. I., Lewis J. (2013): Thinking laterally about neurodegenerative proteinopathies. J. Clin. Invest. **123,** 1847–1855
http://dx.doi.org/10.1172/JCI66029

Gomez-Ramos A., Diaz-Hernandez M., Cuadros R., Hernandez F., Avila J. (2006): Extracellular tau is toxic to neuronal cells. FEBS Lett. **580,** 4842–4850
http://dx.doi.org/10.1016/j.febslet.2006.07.078

Gong B., Leznik E. (2007): The role of ubiquitin C-terminal hydrolase L1 in neurodegenerative disorders. Drug News Perspect. **20,** 365–370
http://dx.doi.org/10.1358/dnp.2007.20.6.1138160

Goryunov D., Liem R. K. (2007): CHIP-ping away at tau. J. Clin. Invest. **117,** 590–592
http://dx.doi.org/10.1172/JCI31505

Gregori L., Poosch M. S., Cousins G., Chau V. (1990): A uniform isopeptide-linked multiubiquitin chain is sufficient to target substrate for degradation in ubiquitin-mediated proteolysis. J. Biol. Chem. **265,** 8354–8357

Grune T., Botzen D., Engels M., Voss P., Kaiser B., Jung T., Grimm S., Ermak G., Davies K. J. (2010): Tau protein degradation is catalyzed by the ATP/ubiquitin-independent 20S proteasome under normal cell conditions. Arch. Biochem. Biophys. **500,** 181–188
http://dx.doi.org/10.1016/j.abb.2010.05.008

Han D. H., Na H. K., Choi W. H., Lee J. H., Kim Y. K., Won C., Lee S. H., Kim K. P., Kuret J., Min D. H., Lee M. J. (2014): Direct cellular delivery of human proteasomes to delay tau aggregation. Nat. Commun. **5,** 5633
http://dx.doi.org/10.1038/ncomms6633

Hardy J., Lewis P., Revesz T., Lees A., Paisan-Ruiz C. (2009): The genetics of Parkinson's syndromes: a critical review. Curr. Opin. Genet. Dev. **19,** 254–265
http://dx.doi.org/10.1016/j.gde.2009.03.008

Hartl F. U., Hayer-Hartl M. (2009): Converging concepts of protein folding in vitro and in vivo. Nat. Struct. Mol. Biol. **16,** 574–581
http://dx.doi.org/10.1038/nsmb.1591

Hartson S. D., Thulasiraman V., Huang W., Whitesell L., Matts R. L. (1999): Molybdate inhibits hsp90, induces structural changes in its C-terminal domain, and alters its interactions with substrates. Biochemistry **38,** 3837–3849
http://dx.doi.org/10.1021/bi983027s

Hendil K. B., Khan S., Tanaka K. (1998): Simultaneous binding of PA28 and PA700 activators to 20 S proteasomes. Biochem. J. **332,** 749–754

Heride C., Urbe S., Clague M. J. (2014): Ubiquitin code assembly and disassembly. Curr. Biol. **24,** R215–220
http://dx.doi.org/10.1016/j.cub.2014.02.002

Hershko A., Ciechanover A. (1998): The ubiquitin system. Annu. Rev. Biochem. **67,** 425–479
http://dx.doi.org/10.1146/annurev.biochem.67.1.425

Ho S. W., Tsui Y. T., Wong T. T., Cheung S. K., Goggins W. B., Yi L. M., Cheng K. K., Baum L. (2013): Effects of 17-allylamino-17-demethoxygeldanamycin (17-AAG) in transgenic mouse models of frontotemporal lobar degeneration and Alzheimer's disease. Transl. Neurodegener. **2,** 24
http://dx.doi.org/10.1186/2047-9158-2-24

Huang L., Chen C. H. (2009): Proteasome regulators: activators and inhibitors. Curr. Med. Chem. **16,** 931–939
http://dx.doi.org/10.2174/092986709787581860

Huang L., Ho P., Chen C. H. (2007): Activation and inhibition of the proteasome by betulinic acid and its derivatives. FEBS Lett. **581,** 4955–4959
http://dx.doi.org/10.1016/j.febslet.2007.09.031

Imai Y., Soda M., Hatakeyama S., Akagi T., Hashikawa T., Nakayama K. I., Takahashi R. (2002): CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. Mol. Cell **10,** 55–67
http://dx.doi.org/10.1016/S1097-2765(02)00583-X

Iqbal K., Liu F., Gong C.X., Alonso Adel C., Grundke-Iqbal I. (2009). Mechanisms of tau-induced neurodegeneration. Acta Neuropathol. **118,** 53–69
http://dx.doi.org/10.1007/s00401-009-0486-3

Jellinger K. A. (2011): Interaction between alpha-synuclein and other proteins in neurodegenerative disorders. ScientificWorldJournal **11,** 1893–1907
http://dx.doi.org/10.1100/2011/371893

Jez J. M., Chen J. C., Rastelli G., Stroud R. M., Santi D. V. (2003): Crystal structure and molecular modeling of 17-DMAG in complex with human Hsp90. Chem. Biol. **10,** 361–368
http://dx.doi.org/10.1016/S1074-5521(03)00075-9

Jhaveri K., Ochiana S. O., Dunphy M. P., Gerecitano J. F., Corben A. D., Peter R. I., Janjigian Y. Y., Gomes-DaGama E. M., Koren J. 3rd, Modi S., Chiosis G. (2014): Heat shock protein 90

inhibitors in the treatment of cancer: current status and future directions. Expert Opin. Investig. Drugs **23,** 611–628 http://dx.doi.org/10.1517/13543784.2014.902442

Karpov D. S., Tutyaeva V. V., Karpov V. L. (2008): Mapping of yeast Rpn4p transactivation domains. FEBS Lett. **582,** 3459–3464 http://dx.doi.org/10.1016/j.febslet.2008.09.010

Katsiki M., Chondrogianni N., Chinou I., Rivett A. J., Gonos E. S. (2007): The olive constituent oleuropein exhibits proteasome stimulatory properties in vitro and confers life span extension of human embryonic fibroblasts. Rejuvenation Res. **10,** 157–172 http://dx.doi.org/10.1089/rej.2006.0513

Keck S., Nitsch R., Grune T., Ullrich O. (2003): Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. J. Neurochem. **85,** 115–122 http://dx.doi.org/10.1046/j.1471-4159.2003.01642.x

Keller J. N., Hanni K. B., Markesbery W. R. (2000): Impaired proteasome function in Alzheimer's disease. J. Neurochem. **75,** 436–439 http://dx.doi.org/10.1046/j.1471-4159.2000.0750436.x

Khan U. A., Liu L., Provenzano F. A., Berman D. E., Profaci C. P., Sloan R., Mayeux R., Duff K. E., Small S. A. (2014): Molecular drivers and cortical spread of lateral entorhinal cortex dysfunction in preclinical Alzheimer's disease. Nat. Neurosci. **17,** 304–311 http://dx.doi.org/10.1038/nn.3606

Kimura Y., Tanaka K. (2010): Regulatory mechanisms involved in the control of ubiquitin homeostasis. J. Biochem. **147,** 793–798 http://dx.doi.org/10.1093/jb/mvq044

Kontsekova E., Zilka N., Kovacech B., Novak P., Novak M. (2014): First-in-man tau vaccine targeting structural determinants essential for pathological tau-tau interaction reduces tau oligomerisation and neurofibrillary degeneration in an Alzheimer's disease model. Alzheimers Res. Ther. **6,** 44 http://dx.doi.org/10.1186/alzrt278

Koren J. 3rd, Jinwal U. K., Lee D. C., Jones J. R., Shults C. L., Johnson A. G., Anderson L. J., Dickey C. A. (2009): Chaperone signalling complexes in Alzheimer's disease. J. Cell. Mol. Med. **13,** 619–630 http://dx.doi.org/10.1111/j.1582-4934.2008.00557.x

Kosik K. S., Shimura H. (2005): Phosphorylated tau and the neurodegenerative foldopathies. Biochim. Biophys. Acta **1739,** 298–310 http://dx.doi.org/10.1016/j.bbadis.2004.10.011

Kovacech B., Zilka N., Novak M. (2009): New age of neuroproteomics in Alzheimer's disease research. Cell. Mol. Neurobiol. **29,** 799–805 http://dx.doi.org/10.1007/s10571-009-9358-6

Kudo T., Iqbal K., Ravid R., Swaab D.F., Grundke-Iqbal I. (1994): Alzheimer disease: correlation of cerebro-spinal fluid and brain ubiquitin levels. Brain Res. **639,** 1–7 http://dx.doi.org/10.1016/0006-8993(94)91757-4

Kuret J., Chirita C. N., Congdon E. E., Kannanayakal T., Li G., Necula M., Yin H., Zhong Q. (2005): Pathways of tau fibrillization. Biochim. Biophys. Acta **1739,** 167–178 http://dx.doi.org/10.1016/j.bbadis.2004.06.016

Kwak M. K., Huang B., Chang H., Kim J. A., Kensler T. W. (2007): Tissue specific increase of the catalytic subunits of the 26S proteasome by indirect antioxidant dithiolethione in mice: enhanced activity for degradation of abnormal protein. Life Sci. **80,** 2411–2420 http://dx.doi.org/10.1016/j.lfs.2007.04.014

Leal G., Afonso P. M., Salazar I. L., Duarte C. B. (2014): Regulation of hippocampal synaptic plasticity by BDNF. Brain Res. (in press) http://dx.doi.org/10.1016/j.brainres.2014.10.019

Lee M. J., Lee J. H., Rubinsztein D. C. (2013): Tau degradation: the ubiquitin-proteasome system versus the autophagy-lysosome system. Prog. Neurobiol. **105,** 49–59 http://dx.doi.org/10.1016/j.pneurobio.2013.03.001

Lee S., Kim J., Seo S. G., Choi B. R., Han J. S., Lee K. W. (2014): Sulforaphane alleviates scopolamine-induced memory impairment in mice. Pharmacol. Res. **85,** 23–32 http://dx.doi.org/10.1016/j.phrs.2014.05.003

Lehman N. L. (2009): The ubiquitin proteasome system in neuropathology. Acta Neuropathol. **118,** 329–347 http://dx.doi.org/10.1007/s00401-009-0560-x

Li J., Soroka J., Buchner J. (2012): The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. Biochim. Biophys. Acta **1823,** 624–635 http://dx.doi.org/10.1016/j.bbamcr.2011.09.003

Lindsten K., de Vrij F. M., Verhoef L. G., Fischer D. F., van Leeuwen F. W., Hol E. M., Masucci M. G., Dantuma N. P. (2002): Mutant ubiquitin found in neurodegenerative disorders is a ubiquitin fusion degradation substrate that blocks proteasomal degradation. J. Cell. Biol. **157,** 417–427 http://dx.doi.org/10.1083/jcb.200111034

Liu A. K., Chang R. C., Pearce R. K., Gentleman S. M. (2015): Nucleus basalis of Meynert revisited: anatomy, history and differential involvement in Alzheimer's and Parkinson's disease. Acta Neuropathol. **129,** 527–540 http://dx.doi.org/10.1007/s00401-015-1392-5

Lloret A., Fuchsberger T., Giraldo E., Vina J. (2015): Molecular mechanisms linking amyloid beta toxicity and Tau hyperphosphorylation in Alzheimers disease. Free Radic. Biol. Med. **83,** 186–191 http://dx.doi.org/10.1016/j.freeradbiomed.2015.02.028

Lu Y., Ansar S., Michaelis M. L., Blagg B. S. (2009): Neuroprotective activity and evaluation of Hsp90 inhibitors in an immortalized neuronal cell line. Bioorg. Med. Chem. **17,** 1709–1715 http://dx.doi.org/10.1016/j.bmc.2008.12.047

Luo W., Dou F., Rodina A., Chip S., Kim J., Zhao Q., Moulick K., Aguirre J., Wu N., Greengard P., Chiosis G. (2007): Roles of heat-shock protein 90 in maintaining and facilitating the neurodegenerative phenotype in tauopathies. Proc. Natl. Acad. Sci. USA **104,** 9511–9516 http://dx.doi.org/10.1073/pnas.0701055104

Mak S. K., McCormack A. L., Manning-Bog A. B., Cuervo A. M., Di Monte D. A. (2010): Lysosomal degradation of alpha-synuclein in vivo. J. Biol. Chem. **285,** 13621–13629 http://dx.doi.org/10.1074/jbc.M109.074617

Mao I., Liu J., Li X., Luo H. (2008): REGgamma, a proteasome activator and beyond? Cell. Mol. Life Sci. **65,** 3971–3980 http://dx.doi.org/10.1007/s00018-008-8291-z

Martin L., Latypova X., Terro F. (2011): Post-translational modifications of tau protein: implications for Alzheimer's disease. Neurochem. Int. **58,** 458–471

http://dx.doi.org/10.1016/j.neuint.2010.12.023
Martinez-Vicente M., Vila M. (2013): Alpha-synuclein and protein degradation pathways in Parkinson's disease: a pathological feed-back loop. Exp. Neurol. **247,** 308–313 http://dx.doi.org/10.1016/j.expneurol.2013.03.005
McFarland N. R., Dimant H., Kibuuka L., Ebrahimi-Fakhari D., Desjardins C. A., Danzer K. M., Danzer M., Fan Z., Schwarzschild M. A., Hirst W., McLean P. J. (2014): Chronic treatment with novel small molecule Hsp90 inhibitors rescues striatal dopamine levels but not alpha-synuclein-induced neuronal cell loss. PLoS One **9,** e86048 http://dx.doi.org/10.1371/journal.pone.0086048
McNaught K. S., Belizaire R., Isacson O., Jenner P., Olanow C. W. (2003): Altered proteasomal function in sporadic Parkinson's disease. Exp. Neurol. **179,** 38–46 http://dx.doi.org/10.1006/exnr.2002.8050
Morishima-Kawashima M., Hasegawa M., Takio K., Suzuki M., Titani K., Ihara Y. (1993): Ubiquitin is conjugated with amino-terminally processed tau in paired helical filaments. Neuron **10,** 1151–1160 http://dx.doi.org/10.1016/0896-6273(93)90063-W
Neckers L., Workman P. (2012): Hsp90 molecular chaperone inhibitors: are we there yet? Clin. Cancer Res. **18,** 64–76 http://dx.doi.org/10.1158/1078-0432.CCR-11-1000
Nisbet R. M., Polanco J. C., Ittner L. M., Gotz J. (2015): Tau aggregation and its interplay with amyloid-beta. Acta Neuropathol. **129,** 207–220 http://dx.doi.org/10.1007/s00401-014-1371-2
Nonaka T., Hasegawa M. (2009): A cellular model to monitor proteasome dysfunction by alpha-synuclein. Biochemistry **48,** 8014–8022 http://dx.doi.org/10.1021/bi900619j
Novak M. (1994): Truncated tau protein as a new marker for Alzheimer's disease. Acta Virol. **38,** 173–189
Novak M., Wischik C. M., Edwards P., Pannell R., Milstein C. (1989): Characterisation of the first monoclonal antibody against the pronase resistant core of the Alzheimer PHF. Prog. Clin. Biol. Res. **317,** 755–761
Novak M., Kabat J., Wischik C. M. (1993): Molecular characterization of the minimal protease resistant tau unit of the Alzheimer's disease paired helical filament. EMBO J. **12,** 365–370
Opattova A., Filipcik P., Cente M., Novak M. (2013): Intracellular degradation of misfolded tau protein induced by geldanamycin is associated with activation of proteasome. J. Alzheimers Dis. **33,** 339–348
Ortega L., Calvillo M., Luna F., Perez-Severiano F., Rubio-Osornio M., Guevara J., Limon I. D. (2014): 17-AAG improves cognitive process and increases heat shock protein response in a model lesion with Abeta25-35. Neuropeptides **48,** 221–232 http://dx.doi.org/10.1016/j.npep.2014.04.006
Owen B. A., Sullivan W. P., Felts S. J., Toft D. O. (2002): Regulation of heat shock protein 90 ATPase activity by sequences in the carboxyl terminus. J. Biol. Chem. **277,** 7086–7091 http://dx.doi.org/10.1074/jbc.M111450200
Pajonk F., McBride W. H. (2001): Ionizing radiation affects 26s proteasome function and associated molecular responses, even at low doses. Radiother. Oncol. **59,** 203–212 http://dx.doi.org/10.1016/S0167-8140(01)00311-5
Panaretou B., Prodromou C., Roe S. M., O'Brien R., Ladbury J. E., Piper P. W., Pearl L. H. (1998): ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. EMBO J. **17,** 4829–4836 http://dx.doi.org/10.1093/emboj/17.16.4829
Park H. M., Kim J. A., Kwak M. K. (2009): Protection against amyloid beta cytotoxicity by sulforaphane: role of the proteasome. Arch. Pharm. Res. **32,** 109–115 http://dx.doi.org/10.1007/s12272-009-1124-2
Petrucelli L., O'Farrell C., Lockhart P. J., Baptista M., Kehoe K., Vink L., Choi P., Wolozin B., Farrer M., Hardy J., Cookson M. R. (2002): Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. Neuron **36,** 1007–1019 http://dx.doi.org/10.1016/S0896-6273(02)01125-X
Petrucelli L., Dickson D., Kehoe K., Taylor J., Snyder H., Grover A., De Lucia M., McGowan E., Lewis J., Prihar G. et al. (2004): CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum. Mol. Genet. **13,** 703–714 http://dx.doi.org/10.1093/hmg/ddh083
Pickart C. M., Eddins M. J. (2004): Ubiquitin: structures, functions, mechanisms. Biochim. Biophys. Acta **1695,** 55–72 http://dx.doi.org/10.1016/j.bbamcr.2004.09.019
Poppek D., Keck S., Ermak G., Jung T., Stolzing A., Ullrich O., Davies K. J., Grune T. (2006): Phosphorylation inhibits turnover of the tau protein by the proteasome: influence of RCAN1 and oxidative stress. Biochem. J. **400,** 511–520 http://dx.doi.org/10.1042/BJ20060463
Prabha C. R., Mukherjee S., Raman R., Kulkarni S. (2012): The ends and means of artificially induced targeted protein degradation. Appl. Microbiol. Biotechnol. **96,** 1111–1123 http://dx.doi.org/10.1007/s00253-012-4471-8
Pratt W. B., Toft D. O. (2003): Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp. Biol. Med. **228,** 111–133
Prince M., Bryce R., Albanese E., Wimo A., Ribeiro W., Ferri C. P. (2013): The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. **9,** 63–75 http://dx.doi.org/10.1016/j.jalz.2012.11.007
Rechsteiner M., Realini C., Ustrell V. (2000): The proteasome activator 11 S REG (PA28) and class I antigen presentation. Biochem. J. **345,** 1–15 http://dx.doi.org/10.1042/0264-6021:3450001
Rideout H. J., Larsen K. E., Sulzer D., Stefanis L. (2001): Proteasomal inhibition leads to formation of ubiquitin/alpha-synuclein-immunoreactive inclusions in PC12 cells. J. Neurochem. **78,** 899–908 http://dx.doi.org/10.1046/j.1471-4159.2001.00474.x
Ristic G., Tsou W. L., Todi S. V. (2014): An optimal ubiquitin-proteasome pathway in the nervous system: the role of deubiquitinating enzymes. Front. Mol. Neurosci. **7,** 72 http://dx.doi.org/10.3389/fnmol.2014.00072
Rivett A. J., Mason G. G., Thomson S., Pike A. M., Savory P. J., Murray R. Z. (1995): Catalytic components of proteasomes and the regulation of proteinase activity. Mol. Biol. Rep. **21,** 35–41 http://dx.doi.org/10.1007/BF00990968
Sahara N., Murayama M., Mizoroki T., Urushitani M., Imai Y., Takahashi R., Murata S., Tanaka K., Takashima A. (2005): In

vivo evidence of CHIP up-regulation attenuating tau aggregation. J. Neurochem. **94,** 1254–1263
http://dx.doi.org/10.1111/j.1471-4159.2005.03272.x

Sahara N., Maeda S., Yoshiike Y., Mizoroki T., Yamashita S., Murayama M., Park J.M., Saito Y., Murayama S., Takashima A. (2007): Molecular chaperone-mediated tau protein metabolism counteracts the formation of granular tau oligomers in human brain. J. Neurosci. Res. **85,** 3098–3108
http://dx.doi.org/10.1002/jnr.21417

Sanchez-Lanzas R., Castano J. G. (2014): Proteins directly interacting with mammalian 20S proteasomal subunits and ubiquitin-independent proteasomal degradation. Biomolecules **4,** 1140–1154
http://dx.doi.org/10.3390/biom4041140

Sarraf S. A., Raman M., Guarani-Pereira V., Sowa M. E., Huttlin E. L., Gygi S. P., Harper J. W. (2013): Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. Nature **496,** 372–376
http://dx.doi.org/10.1038/nature12043

Schmidt M., Finley D. (2014): Regulation of proteasome activity in health and disease. Biochim. Biophys. Acta **1843,** 13–25
http://dx.doi.org/10.1016/j.bbamcr.2013.08.012

Seo H., Sonntag K. C., Kim W., Cattaneo E., Isacson O. (2007): Proteasome activator enhances survival of Huntington's disease neuronal model cells. PLoS One **2,** e238
http://dx.doi.org/10.1371/journal.pone.0000238

Shimamura T., Shapiro G. I. (2008): Heat shock protein 90 inhibition in lung cancer. J. Thorac. Oncol. **3,** S152–159
http://dx.doi.org/10.1097/JTO.0b013e318174ea3a

Shimura H., Miura-Shimura Y., Kosik K. S. (2004): Binding of tau to heat shock protein 27 leads to decreased concentration of hyperphosphorylated tau and enhanced cell survival. J. Biol. Chem. **279,** 17957–17962
http://dx.doi.org/10.1074/jbc.M400351200

Skaar J. R., Pagan J. K., Pagano M. (2014): SCF ubiquitin ligase-targeted therapies. Nature reviews. Drug Discovery **13,** 889–903
http://dx.doi.org/10.1038/nrd4432

Snyder H., Mensah K., Theisler C., Lee J., Matouschek A., Wolozin B. (2003): Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. J. Biol. Chem. **278,** 11753–11759
http://dx.doi.org/10.1074/jbc.M208641200

Spillantini M. G., Schmidt M. L., Lee V. M., Trojanowski J. Q., Jakes R., Goedert M. (1997): Alpha-synuclein in Lewy bodies. Nature **388,** 839–840
http://dx.doi.org/10.1038/42166

Sugino E., Nishiura C., Minoura K., In Y., Sumida M., Taniguchi T., Tomoo K., Ishida T. (2009): Three-/four-repeat-dependent aggregation profile of tau microtubule-binding domain clarified by dynamic light scattering analysis. Biochem. Biophys. Res. Commun. **385,** 236–240
http://dx.doi.org/10.1016/j.bbrc.2009.05.047

Tan Z., Sun X., Hou F. S., Oh H. W., Hilgenberg L. G., Hol E. M., van Leeuwen F. W., Smith M. A., O'Dowd D. K., Schreiber S. S. (2007): Mutant ubiquitin found in Alzheimer's disease causes neuritic beading of mitochondria in association with neuronal degeneration. Cell Death Differ. **14,** 1721–1732
http://dx.doi.org/10.1038/sj.cdd.4402180

Tortosa E., Santa-Maria I., Moreno F., Lim F., Perez M., Avila J. (2009): Binding of Hsp90 to tau promotes a conformational change and aggregation of tau protein. J. Alzheimers Dis. **17,** 319–325

Tseng B. P., Green K. N., Chan J. L., Blurton-Jones M., LaFerla F. M. (2008): Abeta inhibits the proteasome and enhances amyloid and tau accumulation. Neurobiol. Aging **29,** 1607–1618
http://dx.doi.org/10.1016/j.neurobiolaging.2007.04.014

Uryu K., Richter-Landsberg C., Welch W., Sun E., Goldbaum O., Norris E. H., Pham C. T., Yazawa I., Hilburger K., Micsenyi M. et al. (2006): Convergence of heat shock protein 90 with ubiquitin in filamentous alpha-synuclein inclusions of alpha-synucleinopathies. Am. J. Pathol. **168,** 947–961
http://dx.doi.org/10.2353/ajpath.2006.050770

Ustrell V., Hoffman L., Pratt G., Rechsteiner M. (2002): PA200, a nuclear proteasome activator involved in DNA repair. EMBO J. **21,** 3516–3525
http://dx.doi.org/10.1093/emboj/cdf333

van Leeuwen F. W., de Kleijn D. P., van den Hurk H. H., Neubauer A., Sonnemans M. A., Sluijs J. A., Koycu S., Ramdjielal R. D., Salehi A., Martens G. J. et al. (1998): Frameshift mutants of beta amyloid precursor protein and ubiquitin-B in Alzheimer's and Down patients. Science **279,** 242–247
http://dx.doi.org/10.1126/science.279.5348.242

Vilchez D., Saez I., Dillin A. (2014): The role of protein clearance mechanisms in organismal ageing and age-related diseases. Nat. Commun. **5,** 5659
http://dx.doi.org/10.1038/ncomms6659

Vissers J. H., Nicassio F., van Lohuizen M., Di Fiore P. P., Citterio E. (2008): The many faces of ubiquitinated histone H2A: insights from the DUBs. Cell Div. **3,** 8
http://dx.doi.org/10.1186/1747-1028-3-8

Wandinger S. K., Richter K., Buchner J. (2008): The Hsp90 chaperone machinery. J. Biol. Chem. **283,** 18473–18477
http://dx.doi.org/10.1074/jbc.R800007200

Wang J. Z., Xia Y. Y., Grundke-Iqbal I., Iqbal K. (2013): Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. J. Alzheimers Dis. **33** (Suppl 1), S123–139

Wang L., Xie C., Greggio E., Parisiadou L., Shim H., Sun L., Chandran J., Lin X., Lai C., Yang W. J. et al. (2008): The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2. J. Neurosci. **28,** 3384–3391
http://dx.doi.org/10.1523/JNEUROSCI.0185-08.2008

Wang Y., Mandelkow E. (2012): Degradation of tau protein by autophagy and proteasomal pathways. Biochem. Soc. Trans. **40,** 644–652
http://dx.doi.org/10.1042/BST20120071

Wang Y., Martinez-Vicente M., Kruger U., Kaushik S., Wong E., Mandelkow E. M., Cuervo A. M., Mandelkow E. (2009). Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. Hum. Mol. Genet. **18,** 4153–4170
http://dx.doi.org/10.1093/hmg/ddp367

Watanabe N., Yamada S. (1996): Activation of 20S proteasomes from spinach leaves by fatty acids. Plant Cell Physiol. **37,** 147–151

http://dx.doi.org/10.1093/oxfordjournals.pcp.a028925
Wilk S., Chen W. E. (1997): Synthetic peptide-based activators of the proteasome. Mol. Biol. Rep. **24,** 119–124 http://dx.doi.org/10.1023/A:1006851428691
Zilka N., Filipcik P., Koson P., Fialova L., Skrabana R., Zilkova M., Rolkova G., Kontsekova E., Novak M. (2006): Truncated tau from sporadic Alzheimer's disease suffices to drive neurofibrillary degeneration in vivo. FEBS Lett. **580,** 3582–3588 http://dx.doi.org/10.1016/j.febslet.2006.05.029

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