Minireview

The role of the ubiquitin proteasome system in Alzheimer's disease

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Abstract

Today, Alzheimer's disease (AD) is one of the most important age-related neurodegenerative diseases, but its etiology remains still unknown. Since the discovery that the hallmark structures of this disease i.e. the formation of amyloid fibers could be the product of ubiquitin-mediated protein degradation defects, it has become clear that the ubiquitin–proteasome system (UPS), usually essential for protein repair, turnover and degradation, is perturbed in this disease. Different aspects of normal and pathological aging are discussed with respect to protein repair and degradation via the UPS, as well as consequences of a deficit in the UPS in AD. Selective protein oxidation may cause protein damage, or protein mutations may induce a dysfunction of the proteasome. Such events eventually lead to activation of cell death pathways and to an aberrant aggregation or incorporation of ubiquitinated proteins into hallmark structures. Aggresome formation is also observed in other neurodegenerative diseases, suggesting that an activation of similar mechanisms must occur in neurodegeneration as a basic phenomenon. It is essential to discuss therapeutic ways to investigate the UPS dysfunction in the human brain and to identify specific targets to hold or stop cell decay.

Keywords: aging, Alzheimer's disease, oxidation, proteomics, ubiquitination

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Aging and Alzheimer's disease

Normal brain aging is characterized by numerous physiological, structural, functional and neurocognitive changes with a large variability among the aging population. It is characterized by brain volume loss (14% atrophy), especially in the prefrontal cortex and hippocampus area, an increase in the cerebrospinal fluid space, i.e. increase in ventricular volume, and loss of 10% of neurons and up to 20% of synapses.[1–3] Alterations in the generation of immunoglobulins and decreased immune response reduce adaptive defense mechanisms to novel pathogens in the elderly.[4,5] Brain aging is also characterized by cognitive decline, arithmetic/numerical disability, a reduction in perceptual speed, and verbal, semantic and episodic memory loss.[2,3] The term 'successful aging' was first used in 1961 by Motta *et al.*,[6] and describes the absence of significant disease and disabilities, maintenance of a high level of physical and cognitive functions and the preservation of social and productive activities. However, in the very old, only 1.7% of centenarians maintain their independence and functional integrity, while 72.6% are severely dependent.[6]

A major challenge is how to distinguish between normal and pathological aging;[3] mainly Alzheimer's disease (AD). Therefore, identification of cognitive domains that are affected in early AD is quite important.[7] Memory has many facets which are essential for adaptation and strategies, and relate to specific brain areas.[8] These include episodic memory (personally experienced events in a particular temporal and spatial context) in the hippocampus, prefrontal cortex and diencephalon; semantic memory (knowledge of public events, words and concepts) found in the anterior temporal lobe and various neocortical areas; executive functions including coordination of multiple cognitive processes, and are typical for the prefrontal cortex; and working memory (short-term memory) which is attributed to prefrontal cortex. To retard early mental decline, it is essential to have cognitive measures that allow physicians to monitor decline and to evaluate receptiveness for specific treatments.[9,10] These may take the shape of psychological tests, or functional magnetic resonance imaging (MRI) as a predictor of risk of incident,[11] while an age-related mental decline may be slowed down with specific nutrients,[12–16] or a change in lifestyle.[17–19]

At a cellular level, a continuous turnover of intracellular proteins is essential for the maintenance of homeostasis

and for the regulation of multiple cellular functions. There are two main intracellular proteolytic systems: the lysosomal and the ubiquitin–proteasome system (UPS). During oxidative stress, autophagy via chaperone-mediated process may occur, as well as cell activation of repair mechanisms, or protein removal via lysosomes or the UPS.[20] With aging, these mechanisms are characterized by a decrease of protein repair and an increased formation of aggresomes.[21] In brain aging this is seen as neuronal accumulation of lipofuscin, also called dark fat, aging pigment or toxic waste.[22] Lipofuscin contains peroxidised proteins and lipids, pointing to an increasing failure of cells to eliminate such oxidation products.[2]

AD is an age-related neurodegenerative disease, with typical pathological hallmarks such as neurofibrillary tangles (NFT) and senile plaques (SPs). Several excellent reviews summarize the 100 years of AD research and discuss the variations in phenotype, the different cellular and molecular mechanisms and the various hypotheses to explain the disease.[23,24] It is important to also recognize that numerous variants of dementia exist. A small proportion of patients with AD show genetic mutations in the amyloid precursor protein (APP) or in presenilins 1 and 2. In addition, a specific ApoE4 isoform represents a risk factor. Familial AD is based on specific mutations that are transmitted to offspring and represent a minor percentage of cases. The majority of AD cases are sporadic and with a late-onset (after 65 y of age). However, the etiology of the disease remains to be uncovered.

It is important to recognize that memory circuits contain several structures, and a degeneration of any of these structures may affect memory functions, but cognitive deficits may express differently with different symptoms,[9,10] as in AD, fronto-temporal dementia (FTD), corticobasal degeneration or in other forms of dementia. There is therefore a need to identify the disease as early as possible, in order to prime specific medication or to follow progression and responses to specific treatments.

Different hypotheses to explain the etiology of the disease

A recent overview gives a detailed insight into the different mechanisms involved.[25] Yet the links between the different hypotheses and causal consequences need to be further explored. One of the first hypotheses put forward was a deficit in acetylcholine transmission. In a review publication, molecular and cellular mechanisms that include acetylcholin esterases and inflammatory processes were discussed to explain the formation of hallmark structures.[26] Tau proteins with aberrant phosphorylation are major constituents of paired helical filaments (PHFs)[27] and their number correlates with the cognitive status, while the amyloid load does not.[28] However, in some rare cases of AD, only SPs may be found. This suggests that several pathological mechanisms may be involved. The ß-APPs, presenilins and secretases play a major role in the formation of toxic Aß cleavage products and in the formation of amyloid plaques and PHFs.[29,30] A potential influence may also be inflammation of brain tissue with a decrease in the blood–brain barrier function and a change in the humoral immunity.[31–34] Inflammation is also a mediator of oxidative stress and UPS dysfunction.[35] Genetic risk factors, such as ApoE4 polymorphism, are determinant for life expectancy and development of Alzheimer's pathology.[36] Furthermore, tau deposits are not exclusive to AD, but are found in a variety of diseases such as Parkinsonism, corticobasal degeneration, Down's syndrome, FTD, Pick's disease and many more, termed tauopathies.[27] The morphological formations such as tangles or SPs may just represent the tip of the iceberg, caused by more severe metabolic changes.

In recent years, the role of ubiquitin (UBB) and protein degradation was also investigated in relation to AD. In the same year, two groups identified UBB in tangles.[37,38] Since then, both AD and the ubiquitin–proteoasome have been extensively investigated. Here, we will discuss some mechanisms of the UPS that are typical for AD and discuss factors that may lead to neurodegeneration.

Stress, oxidation and nitrosylation in aging and disease

One of the factors that lead to ubiquitination is protein damage due to oxidative stress and the formation of free radical oxygen species (ROS); yet it is still unclear how much oxidation damage is needed to induce ubiquitination. Most likely, irreversible oxidation may activate chaperones and the UPS to induce protein repair of misfolded proteins or lead to ubiquitination and protein degradation. During aging, mitochondria are affected and increasingly produce ROS. In particular, the respiratory chain in mitochondria is strongly linked to the production of ROS, protein dysfunction, apoptosis, necrosis, aging and disease.[39,40] Protein oxidation leads to a change in protein conformation and function and chaperones may sense such changes and in turn activate the proteoasome system as a quality control system. Depending on the degree of oxidation, irreversible oxidation and loss of protein function may lead to degradation and/or accumulation of damaged proteins and to an aggresome formation.[41]

There are several lines of defense mechanisms against excessive oxidation, such as antioxidants (vitamin C, glutathione, lipoic acids, flavonoids, etc.), enzymatic antioxidants (sodium dismutases, catalases, peroxidases, peroxiredoxins, etc.) and protein repair systems (thioredoxin reductase, glutathione reductase, proteasome, etc.).

Several proteomic studies have identified oxidized proteins in AD that belong to abundant proteins such as cytoskeletal proteins or that are involved in metabolism or belong to the UPS themselves.[42–48] Among the proteins are glial fibrillary acidic protein (GFAP,) heat-shock proteins, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1), enolase, ATPase and carboxy terminus of heat-shock cognate (hsc)70 interacting protein (CHIP). Such aberrantly regulated proteins were also found in FTD and are not exclusive to AD pathology.[49] Among oxidative stress, nitrosylation seems to play an essential role since nitric oxide (NO) is a regulator of the UPS;[41] furthermore, GAPDH is a key enzyme to control glycolysis, it is linked to the

cytoskeleton and involved in transcription.[50] GAPDH is also a NO stress sensor[51] and so may influence UPS function.

Protein targets of oxidative damage in human neurodegenerative diseases, with abnormal protein aggregates, have been reviewed recently,[52] with special focus on AD.[53] There is consensus between the different approaches and methods, in that oxidative stress signaling may lead to protein damage and includes several pathways and mechanisms, i.e. glycolysis and energy metabolism, electron transport in mitochondria, structural and cytoskeletal proteins, chaperones and stress proteins, as well as the UPS itself. Such changes may reduce cellular function and eventually lead to cell death.

Role of the UPS and neurodegeneration

The proteasome system, its composition and role during aging and in various diseases have been extensively reviewed.[41,54–56] The UPS is a complex enzymatic pathway that ligates UBB, a small and highly conserved protein to other cellular proteins and destines them to degradation. This process consists of four steps shown in Figure 1. Initially the c-terminal end of UBB is activated by ATP-dependent phosphorylation and formation of a thiol ester via an activating enzyme, E1. It is then transferred to a thiol group of an UBB-carrier protein, E2. A ligase, E3, directs UBB from E2 to an ε-amino group of the target protein.[57,58] For degradation by the proteasome system, target proteins need to be polyubiquitinated by the multiubiquitin-chain elongation factor, E4.[59] The polyubiquitinated proteins are then processed by the polyenzymatic proteasome complex, first deubiquitinated and then degraded by the S26 proteasome, a system that is composed of various proteasome subunits. The eukaryotic 20S proteasome core complex consists of four heptameric rings comprising two classes of seven non-identical but homologous subunits. The outer rings contain alpha-type subunits with gating function for substrate entrance and product release, while the beta-subunits exhibit peptide hydrolysing activity. The 19S regulatory complex binds flexibly to the 20S catalytic core and consists of six ATPases, forming a ring at the entrance of the core and exerting chaperone-like activity.[60] Interestingly, the 19S regulator subunit 6b is found in NFTs and dystrophic neurites in AD and other tauopathies (Pick bodies in a form of FTD) as well as in amyloid plaques of old controls, but is absent in alpha-synucleinopathies (Lewy body disease) and in young controls.

The UPS is also an important quality control system for damaged or misfolded proteins and depends on UBB as a signaling element. Chaperones are involved in the process of protein folding with a cooperation between chaperones and the UPS, but its function is disturbed in AD.[61,62] Molecular chaperones (hsp70 and hsp40) are the first line of defense against protein misfolding and aggregation, and the UPS is the second line; however, a dysfunction of this system may lead to an accumulation and an aggregation of ubiquitinated proteins.[63]

A significant decrease in the proteasome activity was measured in the hippocampus, parahippocampal and middle temporal gyri, and in the inferior parietal lobule of

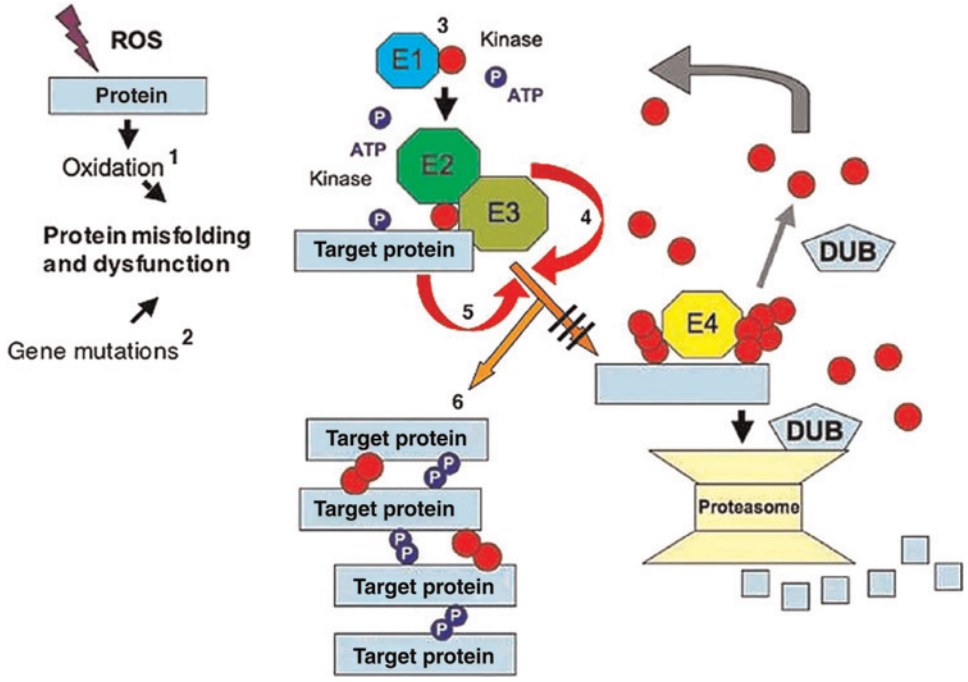

Figure 1 Schematic representation of the mechanisms involved in ubiquitination and disturbed in Alzheimer's disease (AD). See Table 1 for proteins involved in the ubiquitin–proteasome system during AD. Protein damage (1) or protein mutation (2) may lead to a misfolding and protein dysfunction, with a chaperone activation and ubiquitination. (3) Ubiquitin carboxyl-terminal hydrolase isozyme L1 influences ubiquitination. (4) Carboxy terminus of heat-shock cognate (hsc)70 interacting protein, an E3 ligase, together with heat-shock protein hsp70 control ubiquitination. (4) Ubiquitinated tau proteins have an inhitory effect on polyubiquitination of proteins. (5) Mono-ubiquitinated proteins tend to aggregate, and favor paired helical filament formation (A color version of this figure is available in the online journal)

Alzheimer's patients as compared with controls.[64] Brain aging in acquired immunodeficiency syndrome is linked to an increased UBB conjugation and correlates with decreased synaptic proteins but not with the accumulation of amyloid plaques.[65] Several proteins contain UBB-like domains such as ubiquilin, parkin or elongin[66] and the ubiquilin seems to link to AD and to localize with Lewy bodies and tangles. A mutant form of UBB may induce an inhibition of the UPS in AD.[67] De Vrij and colleagues[68] have summarized different proteins that are aberrant or are related to the UPS and to AD such as E1, E2-25K, CHIP, UCH-L1 or tau proteins with an inhibitory effect on the UPS relating to dysfunction of the proteasomal system. This seems a key observation, since some degradation-destined products have a prospective inhibitory effect on protein degradation and therefore result in an accumulation of partially ubiquitinated proteins and aggresome formation instead of being subject to proteolysis.

The UPS dysfunction in AD

Reviews that have summarized and detailed the different aspects of the UPS in disease are numerous.[56,69–71] It is beyond the scope of this Minireview to go into every detail, but we would like to highlight some critical issues that may be relevant for the etiology of AD: namely that protein aggregation and inclusion bodies are characteristic cellular features in neurodegeneration[72] (see also Figure 2).

Oxidative stress signaling may play a key pathogenic role in early AD, with oxidative damage of proteins[40] leading to accumulation instead of degradation. In addition, changes in stress-activated kinases[53] and heat shock proteins or chaperones may influence the proteasome dysfunction.[42] Several proteins are targets for oxidation and consequently ubiquitination. Some recent techniques provide sensitive labeling techniques that allow identification of reduced and oxidized proteins.[34,43–46,48,49] In AD, stress situations such as inflammation, reactive gliosis and oxidative damage may induce protein damage, which in turn results in a tissue reaction such as protein repair or degradation and activation of the UPS and/or abnormal protein aggregation.[47,52]

Some target proteins involved in perturbed UPS and AD

An essential question in proteomic studies is always the relevance of observed proteomic changes. This is even more important when working with human autopsy tissues. Here, we will look at some proteins that may have a key position in the UPS (Table 1). Some members of the UPS themselves may be subject to oxidation or mutations may lead to a protein misfolding and dysfunction, and consequently influence the proteasome. Ubiquinated proteins may also have an inhibitory effect on the UPS and favor protein accumulation instead of protein repair or proteolysis.

In AD, *tau proteins* are major components of PHFs. In 1987 UBB was also identified as a component of PHF.[37,38] This is due to the fact that UBB and ubiquitinated tau are incorporated into PHFs. Tau is subject to a variety of post-translational modifications, hyperphosphorylation, glycosylation, ubiquitination, glycation, polyamination, nitration and proteolysis.[92] Tau is a substrate of the ATP/Mg^{2+}-dependent proteasome system[93] and may inhibit the proteasome and consequently lead to its accumulation.[74] The inhibitory

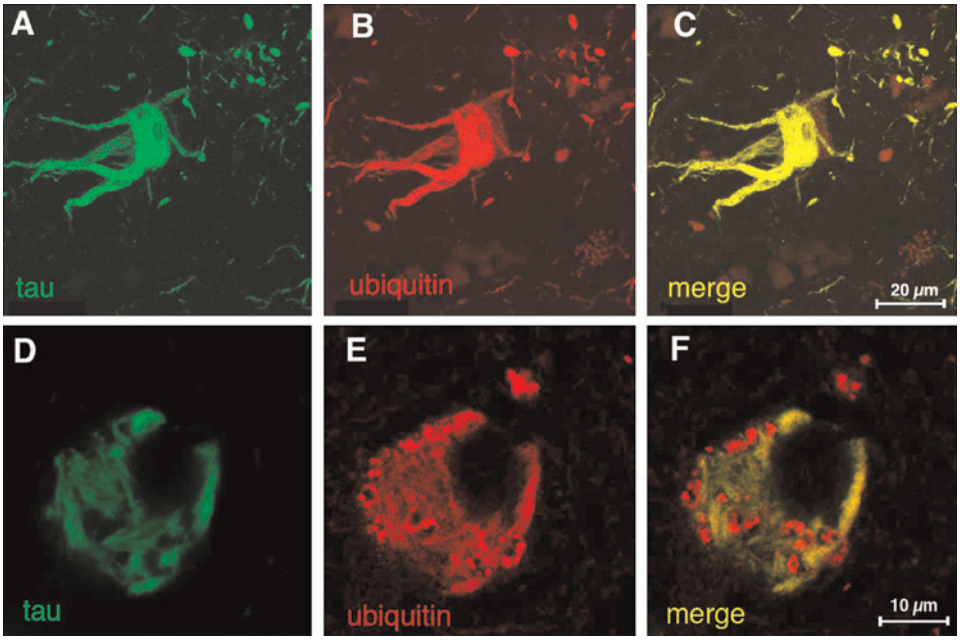

Figure 2 Confocal micrographs show the distribution of phosphorylated tau proteins, typical for paired helical filament (PHF) in green and ubiquitinated proteins in red. Tau proteins were detected by a monoclonal antibody, AD2 and identified with an Oregon green conjugated secondary antibody (panels A and D). The polyclonal antibody against mono-ubiquitinated proteins (Covances) was detected with a Texas red conjugated secondary antibody in red (panels B and E). In merged images (panels C and F), double-labeling shows some co-localization (in yellow) of PHFs in neurons of the entorhinal cortex of an Alzheimer's patient (84-y-old woman with severe Alzheimer's, postmortem delay for autopsy was 13 h). Note the co-localization of tau and ubiquitin in tangles and tau-positive senile plaques. For staining methods, see reference[73]

Table 1 Proteins involved in ubiquitin–proteasome system dysfunction in Alzheimer's disease

Protein	Function	Reference
Tau	Microtubule dynamic, transport, in ubiquitinated form inhibits proteasome, major PHF element, CHIP-dependent, caspase substrate	74–77
CHIP	E3 ligase, control of aggresome solubility	78,79
hsp70 and hsp27	Chaperones, regulate CHIP function	80
cdk5 and GSK3	Trigger binding of tau to hsp70, hsp27 and CHIP	81
BAG-1	Binds to hsp70, when overexpressed inhibits tau degradation	82
UBB +	Gatekeeper function of proteasome, mutations (due to frameshift) have inhibitory function on proteasome	83–85
S6b	Proteasome regulator subunit, found in PHFs	60
UCH-L1	Major control of de-ubiqitination, is reduced in Alzheimer's and Parkinson's disease, influences synaptic and memory functions	86–88
IB-1 (JIP-)	Is also found in PHF and relates to the JNK pathway and apoptosis	89,73
E2-25/Hip-2	Mediator of neurotoxicity, influences JNK patway and apoptosis	90
Phospho-ß-catenin	Induces dysfunction of proteasome	91

UCH-L1, ubiquitin carboxyl-terminal hydrolase isozyme L1; PHF, paired helical filament; CHIP, carboxy terminus of heat-shock cognate (hsc)70 interacting protein; JNK, c-Jun N-terminal kinase; IB-1, islet brain 1; GSK3, glycogen synthase kinase-3

effect of PHF tau on the proteasome activity may explain tau accumulation[75] and a general increase in unbiquitinated proteins.[68,94] PHFs were purified and post-translational modifications were identified by mass spectrometry; tau is ubiquitinated at lys 254, lys 311 and lys 353, as an early event in AD.[76] On the ubiquitin molecule, binding sites are at lys 6, 11 and 48, with major parts at lys 48, known to target the protein to the UPS, while lys 6 inhibits ubiquitin-dependent protein degradation and may favor the formation of PHF instead of tau clearance.[76] In AD, an accumulation of caspase-cleaved tau seems an early event in NFT formation and tau proteins may accumulate together with α-synuclein, as seen also in other neurodegenerative diseases.[77] Apoptosis is induced by the cleavage of cellular substrates by proteases of the caspase family, key enzymes in the cleavage process during neurodegeneration of APP, presenilin and tau proteins.[95] Phosphorylation of tau by Cdk5 and glycogen synthase kinase-3 (GSK3) triggers binding to hsc70 and -27, this implying that phosphorylated-tau may be a substrate for CHIP, an E3 ligase, and that these chaperones may prevent a more toxic conformation of tau.[81]

UBB and UBB-like proteins play a central role in the UPS. Down-regulation of these proteins increases maturation and intracellular trafficking of APP, but does not interfere with secretases, enzymes essential for APP cleavage. Aberrant frameshift proteins as a result of genomic mutations due to dinucleotide deletions, such as found for APP and UBB, may lead to an accumulation in hallmark structures NFTs, SPs and neuropil threads.[84] Ubiquilin 1 (UBB^{+1}) may act as a gatekeeper that controls APP trafficking from the intracellular space to the cell surface.[83] It shows a dose-dependent inhibition of the proteasome activity and seems associated with neurodegenerative diseases, mainly when highly expressed.[96] However, results from a large cohort suggest that a genetic variant has a modest effect on AD risk.[85] An unusual UBB-conjugating enzyme E2-25K/Hip-2 is a mediator of Aß toxicity, and is upregulated in AD. It plays a role in neurotoxicity and proteasome inhibition and is in relation with the upstream apoptosis signal-regulating kinase 1 and c-Jun N-terminal kinase (JNK). The UBB mutant UBB^{+1} is a potent inhibitor of the proteasome and co-localizes with the E2-25K/Hip-2.[90] This suggests that a specific arrangement with heat-shock proteins may be important for aggregation, degradation or protein repair. JNK-interacting protein 1, also called islet brain 1, is an important regulator of the JNK pathway and it is symptomatic that it co-localizes with NFT and amyloid deposits.[73,89] Another protein, phospho-ß-catenin, when accumulating in form of aggresomes may contribute towards a proteasome dysfunction.[91]

UCH-L1 plays a crucial role for the clearance of abnormal proteins. It is one of the most abundant proteins, making 1–2% of soluble proteins, and is exclusively localized in neurons.[86] It has multiple functions: (i) it is a de-ubiquitinating enzyme; (ii) it is a ligase; and (iii) it stabilizes mono-ubiquinated proteins. Down-regulation of UCH-L1 occurs in Parkinson's disease (PD) and AD. Its concentration is inversely proportional to the number of tangles. Results provide evidence for a direct link between oxidative damage to the neuronal UPS and sporadic AD and PD.[87] A substitution of serine by tyrosine at codon 18, exon 3 of the UCH-L1 gene was found to be associated in AD patients of a Chinese Han population, with genotypes more resistant among the female population.[97] UCH-L1 rescues ß-amyloid-induced decrease in synaptic function, as shown in transgenic mice that are overexpressing APP and presenilin. An inhibition of UCH-L1 influences memory functions.[88] A major cellular kinase Akt and microtubule-affinity-regulating kinase (PAR1/MARK2) interact directly and regulate together with hsp90 tau degradation,[98] while hsp70 degrades tau regardless of its phosphorylation state, and does not affect the spatial memory of rats.[99] BAG-1 is a binding partner of hsp70. When overexpressed, it acts rather inhibitory to the degradation of tau and consequently results in an accumulation of tau in neurons and contributes to tangle formation.[82] Interestingly, blueberry-supplemented diet reverses age-related decline in hippocampal hsp70 neuroprotection.[100]

Tools to study UPS dysfunction

Proteomic analysis of human brain tissue and identification of oxidation and ubiquitination may give valuable indications of pathways and tissues that are susceptible to

age- and pathology-related changes. However, an experimental proteomic approach is difficult, as mostly autopsy and not biopsy brain tissue can be obtained, and there is usually some postmortem delay. Protein modifications may depend not only on a variety of parameters, such as age, lifestyle, medication and degree of disease, but also on postmortem oxidative stress, ambient temperature after death to favor proteolysis, or brain regions that were used for analysis. Work in progress indicates a general increase in ubiquitinated proteins in Alzheimer's tissue, while the proportions of ubiquitinated proteins differ between subjects.[101] By using UbiQapture (Enzo Life Sciences, see the protocol of the manufacturer), a matrix with high affinity to bind single UBBs or UBB bound to proteins, ubiquitinated proteins from entorhinal cortex tissue of age-matched control and Alzheimer's tissue were isolated and analyzed by subsequent mass spectrometry.[94] About 600 proteins were identified. Major target proteins are cytoskeletal proteins from all cytoskeletal systems, microfilaments (actins and actin-binding and modifying proteins), intermediate filaments (neurofilaments, GFAP), microtubule proteins with tubulin and major microtubule-associated proteins (MAPs), and membrane-bound brain spectrin. Other proteins are from synapses, cell signaling pathways, glycolysis, ion channels, mitochondrial and redox enzymes, as well as from the UPS and cDNAs and transcription factors.

The use of transgenic animals has increased the knowledge of specific mechanisms such as inflammation, amyloid formation, tauopathy, synaptic deficits or the influence of stress hormones and ultimately allowed this work to lead to clinical trials,[102–104] but a major drawback is that the pathological signs in mice are difficult to correlate with the human pathology.[105–107]

In transgenic CHIP −/− mice, a deletion of this UBB ligase leads to an accumulation but not an aggregation of both endogenous and caspase-3-cleaved tau species. Therefore, polyubiquitination of tau by CHIP may facilitate the formation of insoluble filamentous tau lesions.[78] A major cellular kinase, Akt and microtubule-affinity-regulating kinase (PAR1/MARK2) interact directly and regulate together with hsp90 tau degradation.[98] Therefore, a degradation of proteins depends on many factors that may result in repair, degradation or aggregation of proteins. The regulation of CHIP, its expression and regulation by many proteins makes it an interesting target to regulate PHF formation.

Chaperones are active in neurodegenerative disorders with protein deposits, AD, PD, amyotrophic lateral sclerosis, prion diseases, Huntington's and more. Chaperones favor a refolding and protein repair, while the UPS is essential to reduce the level of abnormal proteins. Accumulation of protein aggregation activates signal transduction pathways that control cell death, such as caspases and the JNK pathway.[73,95] Molecular chaperones may be interesting targets for treatment of neurodegenerative disorders.[108]

Mutant UBB blocks proteasomal degradation in transfected HeLa and SH-SYS5 cells; this mutant is recognized as an UBB fusion degradation proteasome substrate and is ubiquitinated at Lys 29 and Lys 48 and so prevents proteolysis.[109] SH-SYS5 cells were treated with different proteasome inhibitors, MG132, lactacystin and epoxomicin showing that tau is not normally degraded by the proteasome.[110]

Many mutations in proteins lead to protein misfolding and dysfunction. Such proteins are tagged by UBB for the UPS, but instead of being degraded they accumulate and aggravate the UPS. Yet, aggresome formation is recognized as a cytoprotective response serving to sequester potentially toxic misfolded proteins and facilitate their clearance. Inclusion bodies form when COS-7 cells are transfected with huntingtin, parkin, presenilin-1, alpha-synuclein, synphilin-1 or UCH-L1.[111] Cells containing an increased level of aggregates allow to test substances that can dissolve aggregates and to elaborate therapeutic strategies to prevent or alleviate aggregation in neurodegenerative diseases.

Proteasome inhibitors are substances that exert an inhibitory effect on the UPS. They may be valuable tools to study the complex processes of protein ubiquitination, repair or degradation. Several substances were used, such as peptide aldehydes with an inhibitory effect on chymotrypsin-like activity and lysosomal cysteine proteases. Lactacystin and ß-lactone act as a pseudosubstrate and promote neurite growth. Vinyl sulfone acts on ß subunits of the proteasome, while peptide boronate groups bind to active sites of the proteasome.[112] Such inhibitors allowed an identification of proteasome substrates. The UPS is responsible for 90% of the breakdown of abnormal and short-lived proteins (half-life times of hours to days). From total proteins, 80–90% of protein breakdown occurs via the UPS, while 10–20% through the lysosomal proteolysis. A panel of small compounds with inhibitory effect on aggresome formation has been identified.[113]

Phospho-ß-catenin may have a negative effect on the proteasome;[91] it is now possible to use transfected cells to study the formation or dissolution of the proteasomal complex.

A chronic lithium treatment decreased tau lesions by promoting ubiquitination in a mouse model of tauopathy.[114] Lithium is an inhibitor of GSK-3, a major kinase that is responsible for a pathological phosphorylation of tau. Therefore, lithium may affect the formation of pathological structures and so alleviate the burden of AD pathology.

Food for thought

During aging, mitochondrial defects are increasingly occurring and resulting in the formation of ROS and increased protein oxidation. In addition, antioxidative defense mechanisms are reduced, with defective protein repair and UPS dysfunction. This could explain an age-related increase in protein ubiquitination and aggresome formation.[48] Therefore, it seems essential to target events that are upstream of the UPS and that target protein modifications such as excessive oxidation. Nitrones may reduce the free radical-ROS effect on aging in transgenic mice and rats.[115] Age-related decline of cognition can be retarded with specific diets or antioxidants that may represent protective measures to reduce progression of neurodegeneration.[12,14,116] Similarly, reduced antioxidant levels in mild

and severe AD have been shown with a particular plasma composition in centenarians.[13,100,117,118]

In order to target different mechanisms, it is important to understand normal function and their relation to the pathological events.[119] As for protein oxidation, it seems essential to identify the role of different chemicals and food components (fat or antioxidants) and their beneficial or adverse role in the redox pathways and consequently their effect on protein misfolding and aggresome formation. Other factors such as lifestyle and exercise[120] may also help to fight protein oxidation. Reduction in oxidation may also modulate the UPS and eventually prevent or retard the formation of pathological structures and ultimately slow down cognitive decline.

Disclosure: The topic that is addressed here is so vast that it is impossible to cite everybody who has contributed to the progress on Alzheimer's research.

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