

Misfolded protein aggregation and altered cellular pathways in neurodegenerative diseases

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ABSTRACT

Neurodegenerative diseases are estimated by the World Health Organization to be the second leading cause of human death by 2050. They actually are a group of chronic neurological disorders leading to motor, cognitive and sensory impairments in both human and nonhuman species. Despite different in clinical manifestation, prevalence, risk factors, cell types injured and genes hijacked, neurodegenerative disorders are usually associated with the misfolding and aggregation of a distinct protein that accumulates in diverse cellular locations including the nucleus, cytoplasm, plasma membrane and extracellular space. Here we intend to give an overview of the characteristics and features of several pathogenic protein aggregates in disease brains, and introduce some general signaling pathways involved in protein homeostasis with an emphasis on their puzzling roles under the degenerative conditions.

Keywords: Neurodegenerative diseases · Misfolded protein aggregates · Unfolded protein response · Protein clearance pathways · Insulin/IGF/TOR

1. Introduction

Neurodegenerative diseases (NDs) are chronic neurological disorders including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Creutzfeldt-Jakob disease (CJD), Friedreich's ataxia, spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) in humans, as well as scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle and several others in nonhuman species (1-7). Although these diseases are initiated predominantly by aggregations of different misfolded proteins, they all result in gradual and progressive loss of nerve cells in the brain, eventually leading to irreversible disability in learning and memory due to impaired motor, sensory and cognitive systems. Pathologic development of neurodegenerative disorders usually is slow but fatal, requiring the accumulation of pathogenic molecules to exceed some

critical threshold before neurological dysfunction occurs. Many NDs therefore are not evolutionarily selected and associated with the aging process, which provides time to allow the neurogenic symptoms to manifest (8). It was estimated by the World Health Organization that NDs should replace cancer, becoming the second leading cause of human death by 2050, when senior people aged 65 and above reach 17% of the population and over 152 million people are expected to have these dreaded maladies in their later life (9). The numbers underline the urgent need to develop informative molecular diagnostics and effective medical treatment for the public health problem.

While work in the field of neurodegeneration has been sparked by the prevalence of the world-wide epidemic along with increased life expectancy, yet we are only beginning to understand the underlying genetic and cellular mechanisms, and so far limited steps have been made along the path to promising therapeutics for these age-dependent illnesses. In light of this, the goal of this review is to provide an overview of protein misfolding and aggregation in degenerative brain disorders, and focus on debated knowledge regarding the cellular pathways altered in relevance to protein homeostasis under the

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pathological condition. We hope this review will be helpful to inspire new ideas and new discoveries on NDs.

2 Misfolded pathogenic protein aggregates in NDs

Although distinct in clinical manifestation, prevalence, regions of brain targeted and cell types injured, neurodegenerative disorders, when considered at the molecular level, share many common features, among which the progressive accumulation of misfolded pathogenic protein aggregates is believed to be the key event (**Table 1**). The protein aggregates mentioned here can be small and soluble oligomers, large and amorphous assemblies, or highly ordered fibrillary amyloids. A growing body of evidence indicates that these protein agents, such as amyloid β -protein, tau and α -synuclein, when in native states do not exhibit obvious similarities, and in origin can either come from endogenous gene products, or be seeded by an external infectious process, referred to as prion infection.

2.1 Amyloid β -protein ($A\beta$)

Aggregation of misfolded amyloid β -protein ($A\beta$), a secreted peptide derived from an internal domain within the amyloid β -protein precursor (β APP), is an invariant hallmark of all forms of AD (38, 39). It is well known that the β APP protein is normally synthesized, secreted and then efficiently degraded when the internal domain for $A\beta$ is cleaved by α -secretase, a protease, to prevent $A\beta$ formation (40, 41). However, β APP in normal brain can also undergo cleavage in the endoplasmic reticulum (ER)-Golgi secretory pathway by β - and γ -secretase instead of α -secretase to release the amyloidogenic fragment, characterized as a 38- to 48-residue peptide (42-45). Among these toxic peptides, $A\beta_{42}$ is the principal component of amyloid deposits in AD patients as it forms insoluble aggregates much faster than others (46, 47).

It is recognized that the majority of AD cases are sporadic, and only 10% to 20% occur in families (48). Nevertheless, *in vitro* and *in vivo* studies have showed that the underlying genetic factors, whether sporadic or inherited, are aiming to accelerate the accumulation of $A\beta$ neurotoxicity at multiple levels. The first familial mutation discovered was in the β APP gene, near the putative site for γ -secretase cleavage, modifying γ -secretase activity and thereby enhancing only the production of $A\beta_{42}$ (49-51). After that, more inherited β APP variants to facilitate $A\beta$ procession were uncovered (52-54). Subsequent genetic analysis by a large number of AD families also identified mutations in presenilin 1 and 2 genes encoding the catalytic subunits of γ -secretase to increase $A\beta_{42}$ level (55-57). In contrast, apolipoprotein E, a cholesterol transporter binding to $A\beta$, is the only well-established genetic factor associated with sporadic AD through its function to influence the clearance of $A\beta$ in extracellular space (58-60).

2.2 Tau and tauopathies

Tauopathies are a diverse group of neurodegenerations characterized by neurofibrillary tangles (NFTs) composed

of insoluble and hyper-phosphorylated tau proteins in neurons and glia (61). The protein tau, however, naturally is highly soluble and functions as a microtubule (MT)-binding protein to stabilize and promote the assembly of MTs (62). The binding between tau and MT is negatively regulated by the phosphorylation of tau, which is a feature of its pathogenic form (63). In adult human brains, tau is encoded by the *MAPT* gene to generate six isoforms, containing either three or four MT-binding repeats via alternative mRNA splicing (64). It has been proved *in vitro* that the MT-binding repeats are both necessary and sufficient for tau to acquire highly ordered β -sheet structures when it assembles into insoluble NFTs (65). Hence it has been shown that all six isoforms are present and misfolded in disease brains to form a heterogeneous mixture of tau isoforms adopting different conformations, which is probably responsible for the clinical and pathological diversity of tauopathies (66).

As a MT-binding protein, tau is normally considered to function inside a cell, but tau aggregates, likely released from dying or dead neurons, are also detected in the extracellular space where it can be taken up through endocytosis by neighboring cells (34). Once internalized, the small amount of aggregated tau then serves as a seed and transmits a misfolded state specifically to the native tau in healthy cells in a manner similar to prion, which will be discussed later (67). In this way, the disease properties spread from cell to cell along the defined neuroanatomical pathways, causing cellular dysfunctions due to both the physical occupancy of the large tau deposition and the loss of the MT-binding function of tau. Especially the latter, not only disrupts the stabilization of MT cytoskeleton, which is important for the generation and maintenance of neurites, but also suppresses the kinesin-dependent transport of mitochondria, peroxisomes and Golgi-derived vesicles in neurons (68). Shortage of mitochondria and peroxisomes subsequently causes loss of energy production and accumulation of reactive oxygen species, leading to degeneration. In particular, suppression of Golgi-mediated secretion would retain vesicles carrying β APP in the cell body, allowing an enhanced production of toxic $A\beta$ peptides (69, 70).

2.3 α -synuclein (α -syn)

α -synuclein (α -syn) is a phospholipid-binding protein with a chaperone activity to facilitate presynaptic SNARE-complex assembly and thereby regulate neurotransmitter release in the presynaptic terminals (71). In the presence of negatively charged lipids, normal α -syn folds into amphipathic α -helices through its N-terminal repeat region. Missense mutations located in the N-terminal repeats often lead to the conversion of α -helices into β -sheet-rich structures, which ultimately coalesce into characteristic assemblies called Lewy bodies and Lewy neurites in maladies such as PD and Lewy body disease, as well as into glial cytoplasmic inclusions in multiple system atrophy (20, 72-74).

As in the case of tau protein, the neuropathological process of α -syn lesions is also thought to progress via a

Table 1. Misfolding and aggregation of pathogenic proteins identified from NDs.

Misfolded Protein	Gene	Subcellular locations	Diseases	References
Amyloid β	<i>βAPP</i>	ER-Golgi, autophagosome, mitochondria, ES	AD, PD	(10-13)
Androgen receptor	<i>AR</i>	cytosol	SBMA	(14)
Atrophin 1	<i>ATN1</i>	nucleus, cytosol	DRPLA	(15)
Ataxin 1	<i>SCA1</i>	nucleus	SCA	(16)
α -Synuclein	<i>SNCA</i>	nucleus, cytosol, ER, mitochondria, PM, ES	DLB, PD	(2, 17-20)
Fused in sarcoma	<i>FUS</i>	nucleus, cytosol	ALS, FTD	(21)
Huntingtin	<i>HTT</i>	nucleus, cytosol	HD	(22)
Prion protein	<i>PRNP</i>	nucleus, cytosol	CJD, Kuru, BSE, CWD, Scrapie	(7, 23-26)
Rhodopsin	<i>RHO</i>	ER, PM	ADRP	(27)
Superoxide dismutase 1	<i>SOD1</i>	nucleus, cytosol, ER, mitochondria	ALS	(28)
Tau	<i>MAPT</i>	nucleus, cytosol, ER, Golgi, lysosome, PM, ES	AD, FTD, Pick's disease	(29-35)
TAR DNA-Binding Protein 43	<i>TARDBP</i>	nucleus, cytosol	ALS, FTD	(21, 36, 37)

AD: Alzheimer disease; ADRP: Autosomal dominant retinitis pigmentosa; ALS: Amyotrophic lateral sclerosis; β APP: amyloid β -protein precursor; BSE: Bovine spongiform encephalopathy; CJD: Creutzfeldt-Jakob disease; CWD: Chronic wasting disease; DLB: Dementia with Lewy bodies; DRPLA: Dentatorubral-pallidoluysian atrophy; ER: endoplasmic reticulum; ES: extracellular space; FTD: Frontotemporal dementia; HD: Huntington's disease; MAPT: microtubule-associated protein tau; PD: Parkinson's disease; PM: plasma membrane; SBMA: Spinal and bulbar muscular atrophy; SCA: Spinocerebellar ataxia; SNCA: Synuclein Alpha.

seed-induced conversion among cells along anatomically connected structures in the brain, albeit how pathological α -syn exits cells remains elusive (75). Furthermore, compelling evidence has suggested that abnormal α -syn is frequently co-depositing with other pathogenic proteins like A β and tau, as hybrid polymers initiated by cross-seeding between different types of protein aggregates have been extensively reported in various NDs (76, 77). As a result, the pathological overlap between disease agents in the same patient raises the question of which one is the predominant cause and complicates the diagnosis and treatment for NDs.

2.4 Prion diseases

Prion diseases, such as CJD and Kuru in humans, as well as scrapie and BSE in animals, can arise sporadically, be inherited, or be acquired by infection under natural conditions. The term “prion”, denoting a small proteinaceous infectious particle, was proposed by Stanley Prusiner in 1982 first to describe the scrapie agent that causes a degenerative disorder of the central nervous system in sheep and goats (7). The definition now has been broadened to emphasize the requirement of an unconventional and virus-like protein for infection, which is able to undergo self-replication, similar to nucleic acid molecules, but resistant to procedures with specificity for attacking nucleic acids (78). Hence, it is now widely accepted that the pathogen of prion diseases might not contain any DNA or RNA, unless more sensitive probes are developed.

Although prions are thought to exist in multiple strains composed of different polymeric forms of misfolded proteins to cause phenotypic heterogeneity in various brain disorders, they all arise when normal cellular proteins (PrP^C-Cellular, or PrP^C) misfold and transform into pathogenic prion molecules (conventionally referred to as PrP^{Sc}-Scrapie, or PrP^{Sc}), which are characterized by a high content of β -sheets. Once established in neurons, the disease agent PrP^{Sc} then indefinitely convert more PrP^C into the prion form. Mutations in the gene encoding PrP have been identified prone to develop infectivity spontaneously (79, 80). This perhaps hints a genetic origin of prion diseases, but how pathological transformation occurs when PrP^C binds to PrP^{Sc} is largely unknown. It is predicated that the efficiency of prion conversion could depend on the homology of the primary and secondary structures between PrP^C and PrP^{Sc}, and the architecture of the PrP^C-PrP^{Sc} complex (81). According to studies on different prion strains, it is plausible to suspect that environmental factors may also contribute to the conversion of PrP^C to PrP^{Sc} as non-host factors, such as surface binding and weathering, which are able to alter strain emergence in vitro in a population of prions (82, 83).

Toxic prions have an enhanced tendency to aggregate and form oligomers or amyloid-like fibrils, disrupting normal cellular functions and eventually spreading within the nervous system mainly through the neural connectome (84). Besides cell-to-cell transmission, person-to-person and even cross-species disseminations are suggested by cumulative evidence as cases were reported that people

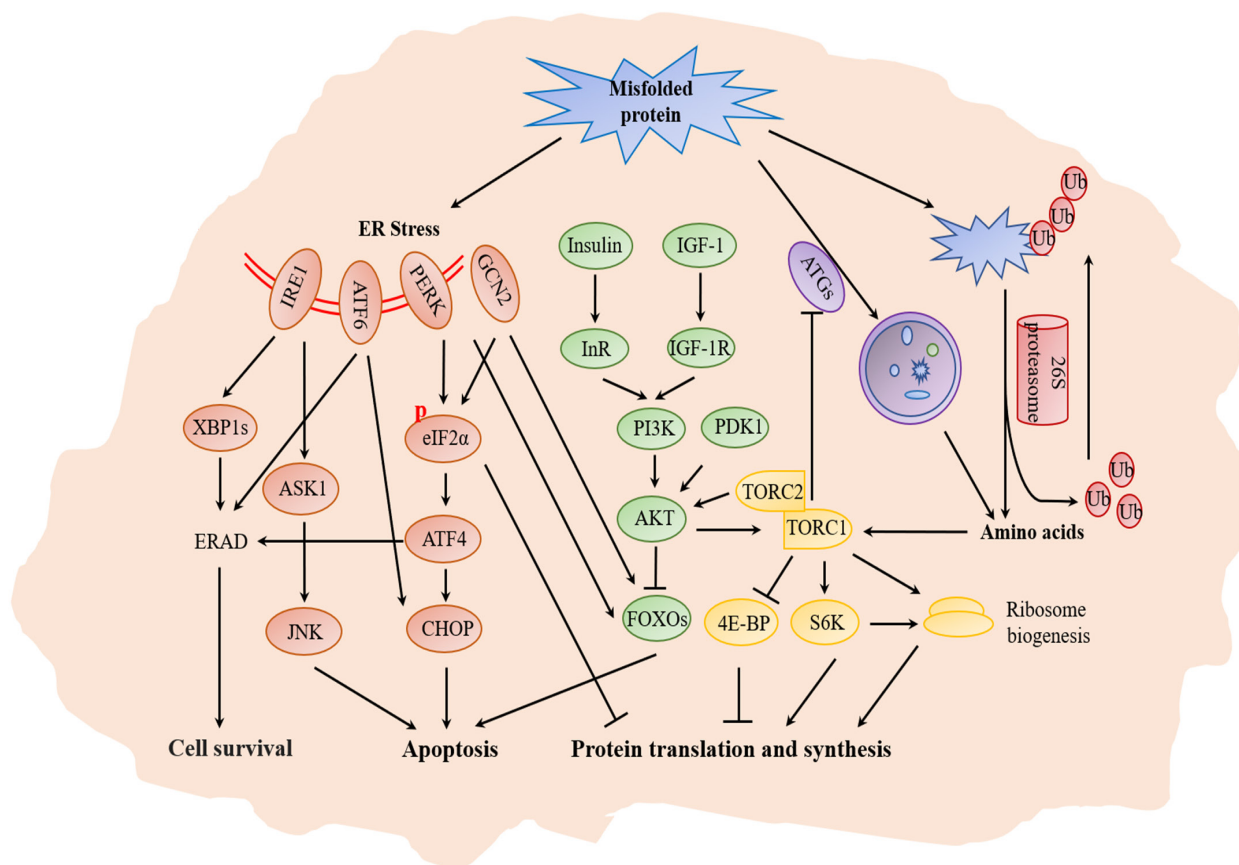


Figure 1. Schematic representation of cellular pathways involved in NDs. Arrows indicate activation, whereas bar-ended lines indicate inhibitory interactions.

with CJD, resulting from consumption of beef prepared from mad cows, transmitted CJD prions to recipients of blood transfusions (23, 85). However, the molecular basis of the intra-species and the inter-species transmissibility of prions remains poorly understood.

2.5 Prion v.s. non-prion

Even though lots of common biological features are shared, we insist to classify prion and non-prion ($A\beta$, tau, α -syn and others) into two groups in this review based on the transmissibility of associated diseases. After all, there is no clinical evidence for the person-to-person transmission of non-prion NDs under normal circumstances. Nevertheless, studies with $A\beta$, tau and α -syn have clearly shown that experimental inoculation with brain homogenates from patients or mouse models of these illnesses could lead to disease pathology in recipient animals in laboratory (86-88). As such, it is highly possible that the definition of prion will be further widened when bioassays are well developed so that the transmissibility of non-prion proteins could be fully appreciated. Yet we sincerely hope the infectious property of non-prion diseases is not true as it should challenge the therapeutic strategies and require implementing more precautions in taking care of ND patients. Also, it is serious that to date there are no effective therapies

available for prion diseases. Approaches have been explored including small compounds, antibiotics, vaccination, antibodies, peptide aptamer and nucleic acid-based agents, but none have prospects for clinical advancement, owing to either inefficacy against prion after onset of symptoms or inadequate brain distribution. It hereby should be pointed out that a breakthrough from clinical trials can only be achieved with the development of a screening test for the early diagnosis of prion diseases (89).

3 Alteration of signaling pathways in cells of NDs

A key question always concerns how the accumulation of distinct disease proteins contributes to the degenerative process. The mechanisms underlying different neurological disorders probably are not exactly the same, but dysregulation of protein homeostasis linked with abnormal aggregates is an almost universal hallmark of ND pathogenesis. In patients, activities of pathways involved in protein synthesis, protein folding, protein degradation and energy supply for proteostasis are altered in cells of the nervous system (Figure 1). However, it is still not completely clear whether these changes play a protective or a toxic role in cell survival.

3.1 ER stress and unfolded protein response

The ER plays a central role in protein quality control to

maintain cellular proteostasis. Membrane and secreted proteins are synthesized, folded and processed in the ER before displayed on the cell surface, or released extracellularly. Misfolded proteins are eliminated via the ER-associated degradation (ERAD) pathways, either the ubiquitin-proteasome system (UPS) or autophagy (also termed as ERAD-I and ERAD-II respectively in some publications), to ensure that only properly folded proteins exit the ER (90). When substrates exhaust the regulatory capacity of ERAD, misfolded proteins accumulate and lead to a stress response called the unfolded protein response (UPR) (91). The UPR is mediated through three principal branches including endoribonuclease IRE1, transcription factor ATF6, and eIF2 α kinases PERK and GCN2. The three signal transducers then regulate the expression of tremendous genes to adapt to the stress or to induce cell apoptosis when the stress cannot be mitigated (92).

In most organisms, ER stress-associated UPR is exacerbated during the aging process (93, 94). The capacity of the ER to prevent aberrant protein dramatically decreases in healthy aging, while the burden of unfolded proteins increases instead. In this scenario, the UPR is known to activate its adaptive programs to alleviate the accumulation of misfolded proteins via halting protein translation, stimulating destruction of abnormal proteins by ERAD, and increasing the production of ER chaperons relevant to protein folding. Upon activation, IRE1 is usually acting as an RNase and mediating the removal of an intron from the *XBPI* mRNA to allow the expression of a functional XBP1 transcription factor (95). The activity of XBP1 is linked to various pro-survival events including transcription of genes involved in protein folding and ERAD (96). ER stress also directly modulates gene expression to promote cytoprotection through the transcription factor ATF6 after it is translocated from the ER to Golgi, where ATF6 is activated by a proteolytic cleavage (97). In addition, the protein translation initiation factor eIF2 α is phosphorylated upon stress to globally attenuate the cap-dependent mRNA translation and prevent overload of newly synthesized proteins into the already stressed ER lumen (98). In contrast, under eIF2 α phosphorylation, translation of a subset of mRNAs, such as transcription factor ATF4 and genes targeted by XBP1 and ATF6, is enhanced to restore homeostasis via upstream open reading frame (99). Phosphorylation of eIF2 α in response to ER stress was initially found to be controlled by auto-phosphorylation of the ER-resident PERK kinase, and it is recognized now to be partially contributed by GCN2 as well (100, 101). Although it remains unclear how the cytoplasmic GCN2 kinase senses ER stress, the redundant regulation of the two eIF2 α kinases was suggested to occur in a tissue dependent manner (102).

ER stress-triggered UPR has been implicated broadly in neurodegeneration. Previous work in a *Drosophila* model of PD showed that accumulation of wild type or missense mutant α -syn led to the hyper-activation of IRE1, and ectopic overexpression of IRE1 was sufficient to induce neuron death, progressive locomotor impairment and

shorter lifespan of flies (103). In brain tissues from both AD and PD patients, a clear increase of PERK and eIF2 α phosphorylation levels was also observed when compared to normal elderly controls by antibody staining (104, 105). Interestingly, the same mammalian PD research and others demonstrated that oral administration of a PERK inhibitor had strong neuroprotective effects on many ND models, implicating the potential use of eIF2 α phosphorylation as therapeutic targets, even though PERK inhibitor itself was found to have strong undesired side effects (105-107). Finally, ATF6 overexpression has been reported recently to reduce misfolded proteins and restore memory in disease animals albeit less is known about the involvement of ATF6 in neurodegenerative disorders (108).

Taken together, all these studies suggest a complicated scenario where the three parallel arms of the UPR, in comparison to its protective function, turn out to have contrasting and even opposite effects, as sustained ER stress, depending on the disease context, shifts the UPR signaling towards induction of apoptosis. Theoretically, the apoptotic effects are tuned through different downstream networks controlled by the same batch of genes, such as the ASK1 (Apoptotic-Signaling Kinase-1)-JNK pathway mediated by IRE1, pro-apoptotic transcriptional factor CHOP activated by ATF6 and ATF4, as well as apoptosis-related transcription factor FoxO3 (also in section 3.5) phosphorylated by PERK and GCN2 (102, 109-111). However, when and how the UPR converts its dual effect under ND conditions of chronic and irreversible ER stress is still incompletely understood.

3.2 Ubiquitin-proteasome system

Coordinated activities of the UPS and autophagy, the two major protein clearance pathways, can be central to prevent the aggregation and toxicity of misfolded-prone proteins, which manifest in a number of neurological disorders. The UPS is a highly selective and tightly regulated pathway for destruction of soluble, unneeded or potentially toxic polypeptides in most cellular compartments (112). Degradation of a protein via the UPS involves two discrete and consecutive steps named conjugation and degradation: the substrate protein is tagged by covalent attachment of multiple ubiquitin molecules to synthesize a proteolytic signal during the conjugation step; thereafter, the polyubiquitinated substrate is chewed up by the 26S proteasome complex with release of free and reusable ubiquitin, which is the degradation step (113).

Accumulation of ubiquitinated proteins has been reported in NDs, and an age- and disease-related decline of UPS activity has also been reported (114-116). In some cases, malfunctions of the UPS have emerged as a primary cause in the pathogenesis of neurodegenerations. In the past two decades, for instance, a direct link between an aberration in the ubiquitin system and the resulting pathology has been studied in PD.

The gene *Parkin* (or *PARK2*) codes for a ubiquitin ligase that ubiquitinates misfolded proteins targeted for proteasome-dependent degradation (117). Various deletion

and point mutations were found in this gene leading to young-onset PD (118, 119). Since then, a broad array of candidate substrates for Parkin has been identified including α -syn and its interacting protein synphilin-1, which are responsible for Lewy-body formation (120, 121). It should be noticed that by recent findings Parkin also ubiquitinates substrates on the outer membrane of mitochondria and through the UPS participates in the elimination of damaged mitochondria, which contributes to neuronal death as well when Parkin is impaired (122).

Besides, aberrations in the UPS have been implicated as a secondary consequence by disease-associated aggregations in many other cases. Cells engineered to produce or infected with unrelated protein aggregates by different research groups were shown to have the UPS stalled and destroyed (123, 124). Bennett et al., 2005 further found that production of protein aggregates specifically targeted to either the nucleus or cytosol led to global impairment of the UPS function in both compartments (125). Although the molecular mechanisms are undetermined, the observation of severe UPS damage in cellular compartments lacking detectable disease agent suggests UPS disruption could be an indirect phenomenon, arguing the toxic gain-of-function mediated by pathogenic protein aggregates in NDs.

3.3 Autophagy

Autophagy (or macro-autophagy) is a bulk clearance pathway whereby misfolded and proteasome-resistant proteins, macromolecules, and damaged or excess organelles are packaged into double-membraned vacuoles called autophagosomes, and then transported along MTs to the lysosome for degradation (126). Autophagy is normally regulated through a series of protein-coding genes defined as autophagy-related genes (ATGs) to constitutively function at a low level (127). Although autophagy in many organisms is induced primarily in adaption to nutrient deprivation, a tight relationship between autophagy and ER homeostasis is confirmed, given that many terms like “ERAD-II”, “ER-quality control autophagy (ERQC)”, “ER-autophagy (ER-phagy)” and “ER-to-lysosome-associated degradation (ERLAD)” have been proposed to delineate variant ER pathways that intersect with the entire or selective autophagy machinery (90, 128-130).

However, the pathological connection between autophagy and neurodegeneration is not simply restricted to the ER, and is much more complex. Experimental result has supported a role for dysfunctional autophagy as a potential causative factor of NDs, since mice deficient for *Atg5* specifically in neural cells develop progressive motor and behavior deficits, accompanied by the accumulation of cytoplasmic inclusion bodies in neurons (131). The absence of *Atg5* suggests the basal activity of autophagy is already essential for preventing the accumulation of abnormal proteins in the nervous system even without expressing any disease-linked mutant proteins. Not surprising that, in the presence of toxic protein aggregates, increased induction of autophagy is relatively frequent,

and substantial benefits to ameliorate neuropathology are often observed with autophagy-inducing agents in a majority of transgenic mouse models of NDs (132, 133).

Yet there are a few exceptions that stimulation of autophagy would become counter-productive when specific stages of autophagy for clearance have been compromised by disease proteins. For example, certain tau isoform has been shown to bind the lysosomal membrane rather than enter the lysosome for degradation (35). In this context, autophagy induction seems to deliver more tau fragments to the lysosome and promote the formation of tau oligomers at the surface of these organelles. Also notably, biochemical experiment implies that A β is generated not only in the ER and Golgi compartments but also in autophagosomes, as purified autophagic vacuoles contain both β APP and highly activated γ -secretase, the protease cleaving β APP to A β (11). Moreover, autophagy is illustrated to influence A β secretion *in vivo* in β APP transgenic mice, where autophagy deficiency reduces extracellular A β plaque burden and leads to aberrant intra-neuronal A β accumulation, contrary to what may be expected if autophagy only cleaned A β (134).

Overall, autophagy responses are generally viewed as neuroprotective, and stimulating the induction of autophagy has therapeutically received great attention. Although consequences of pharmacological modulation of autophagy are still beyond our current knowledge, in specific neurodegenerative disorders where autophagic clearance mechanisms are well-understood, further promotion of autophagy might be the best interventional strategy so far.

3.4 Target of rapamycin (TOR)

The evolutionarily conserved protein kinase TOR has garnered significant attention for its role in neurological diseases. Biochemical purification of TOR-associated proteins has revealed that TOR is present in two complexes, TORC1 and TORC2, with distinct sets of binding partners (135). The two complexes coordinately regulate fundamental cellular behaviors, such as protein synthesis, cytoskeletal organization, cell metabolism, cell proliferation and survival. Compared to TORC1, less is known about TORC2, part of whose function is believed to impact TORC1 through positive and negative feedback mechanisms (136). As such, we will only review the linkage between TORC1 and NDs in this section.

TORC1 and its downstream pathways have been intensively shown to be altered in a variety of neurodegenerations, but the data appear to be extremely conflicting. First of all, TORC1 is a negative regulator of autophagy in response to growth factors, amino acids and cellular energy (137, 138). When TORC1 activity is high, Atg13 undergoes TOR-relied phosphorylation, which blocks autophagosome formation (139). In this circumstance, beneficial effects of removing pathogenic proteins were obtained when using the TOR inhibitor, rapamycin, to induce autophagy in ND models (140). In the case of tauopathies, rapamycin also suppresses TOR-mediated phosphorylation of S6K (ribosomal protein

S6 kinase), and in turn inhibits S6K-catalyzed hyperphosphorylation of tau, which may foster the conversion of tau into its pathogenic form (141). On the other hand, loss of TORC1 signaling has been implied to impair synaptic plasticity and memory storage in animal models of AD, which can be restored through upregulation of TORC1 activity (142). This is most likely because of the function of TORC1 to modulate protein synthesis required for memory consolidation, given that altered translational control has a vital role in memory and cognitive decline (143). Two well-characterized substrates of TORC1 are involved in the initiation of cap-dependent translation of mRNA: 4E-BP (eIF4E-binding protein) and S6K. Phosphorylation of 4E-BP by TORC1 leads to its dissociation from eIF4E and allows the assembly of the translation preinitiation complex (144). S6K, as mentioned earlier, actually is best known for its ability to phosphorylate 40S ribosomal protein S6 and eIF4B, which enhances the association of eIF4B with the translation preinitiation complex (145). Additionally, TORC1 is also a key mediator of ribosome biogenesis, essential for cell growth and survival (146). Taken together, it is reasonable as well that a decrease in TORC1 activity appears to be harmful and correlate with the progression of ND in clinical patients.

How to explain the discrepancy of TORC1 in degenerative disorders? To some extent, there is a chicken-and-egg scenario here: it is difficult to determine whether alteration of TORC1 signaling emerges first, then contributing to neurodegeneration, or whether activity of TORC1 is adjusted by the cell as a secondary consequence, struggling to survive in response to an existing pathological condition. The two models apparently will lead to opposite outcomes, and current information seems to support both in different physiological contexts of NDs. Alternatively, as speculated from “Norambuena A, et al. 2018” and “Polanco JC and Götz J. 2018”, it is where TORC1 is functioning that matters, rather than whether it is up or down (147, 148). In fact, TORC1 has been detected in multiple subcellular compartments, not only in the nucleus, cytoplasm and Golgi, but also located on vacuoles/lysosomes and plasma membrane (149, 150). How the subcellular distribution of TORC1 affects specific cellular responses remains an open question. However, Norambuena A, et al. 2018 found that in the early stages of AD, oligomeric A β would abrogate lysosome-localized TORC1 function by an activation of TORC1 at the plasma membrane, where tau is phosphorylated in a TORC1-dependent manner (147). In light of this, subcellular localization may be an important principle used in AD to enact precise spatial and temporal control of TORC1. It will be intriguing to further investigate whether it also holds true for other degenerative diseases.

3.5 Insulin/insulin-like growth factor (IGF) signaling

The mammalian brain has a high demand for energy. Despite representing only 2% of the total body mass, the brain consumes approximately 25% of the glucose and oxygen used by the body (151). As a matter of fact, nearly

all neurodegenerations have been corroborated to exhibit a crucial metabolic dysfunction that includes altered glucose uptake/utilization and disrupted mitochondrial activity. The insulin/IGF signaling responsive to systemic hormonal cues is the main regulatory network controlling energy metabolism and longevity in multicellular animals (152, 153). Insulin and IGFs, closely related in terms of biological activity, are primarily secreted from different organs, yet both are also locally synthesized in the brain (154). Insulin resistance takes place when cellular responsiveness to insulin/IGFs is compromised, leading to a disturbance in glucose metabolism and energy balance. Strong evidence has underscored that type 2 diabetes and midlife obesity associated with insulin resistance are risk factors for development of dementia, PD, AD and HD (155-158).

However, contradiction appears in literatures when this comes to the level of molecular and cell biology. While some studies reported reduced expression of insulin, IGFs and their receptors in brains of AD and PD by quantitative RT-PCR, more tried to prove elevated insulin/IGFs in the serum and cerebrospinal fluid of patients with neurological diseases, including AD and PD (159-162). What is more controversial is that positive effects have been observed either by decreasing insulin/IGF signaling or by administration of agonists of insulin and IGF-1 in preclinical models (163, 164). Interestingly, an *in vitro* assay has showed that A β in AD is a direct competitive inhibitor on insulin binding to its receptor, indicating insulin resistance perhaps is not simply resulting from the changed amount of pathway components (165). Alternatively, the inconsistency might come from the time point chosen for investigation during the whole disease course. As indicated by a survey based on 3,139 participants for up to 10 years in Rotterdam of Netherlands, the interconnection between insulin metabolism and the clinical manifestation of ND does exist but seems not constant over time (166).

In mammals, both insulin and insulin-like growth factors (IGFs) activate the phosphatidylinositol 3-kinase (PI3K)/AKT pathway through their respective receptors. The protein kinase AKT is recruited to the plasma membrane via phosphatidylinositol-triphosphate (PIP3), which is generated through phosphorylation of PI-4,5-P2 by PI3K. Membrane-recruited AKT then is activated and phosphorylated successively by PDK1 and by TORC2 (153). By monitoring the level of AKT phosphorylation, AKT activity has been implied to be important for neuronal survival and usually is low when cell is insulin resistant (167). Additionally, it has been shown that AKT is able to negatively interact with several pathogenic proteins via different mechanisms, complicating the regulation of AKT in NDs (168). Anyway, a chicken-and-egg analogy could also be used to summarize the interplay between neurodegenerations and AKT, similar to the situations with TORC1.

AKT has a couple of downstream effectors, including TORC1 and FoxOs, the Forkhead box class O transcription factors. Through AKT, TORC1 integrates

information about growth factor signals and nutritional status to adjust cellular proteostasis in conditions such as NDs, which has been discussed in the previous section. In addition to TORC1, AKT also mediates the phosphorylation of FoxO and creates binding sites for 14-3-3 proteins, which promotes the retention of FoxO in the cytoplasm, thereby lowering its activity in the nucleus (169). The evolutionarily conserved FoxO transcription factors are well-known to modulate the expression of genes involved in cell survival, stress response, metabolism and longevity (170). Mammals have four FoxO genes, FoxO1, 3, 4 and 6, which are expressed in the nervous system at different levels with high similarity in their function and regulation (171, 172). The expression of FoxO overall is increasing progressively in aging human and mouse brains. In mice, nervous system specific FoxO1/3/4 loss-of-function accelerates aging-related degeneration followed by motor dysfunction (173). By contrast, overexpression of a constitutively active FoxO3 has pro-apoptotic effects leading to neuronal loss, suggesting that fine-tuning FoxO level is of some importance to neurons. Intriguingly, inhibition of FoxO3 by expressing a dominant negative competitor is absolutely protective when a pathogenic α -syn is co-expressed to induce a disorder condition, highlighting FoxO as a potential target for ameliorating the cytotoxicity of misfolded pathogenic proteins of NDs (174).

4 Conclusions

Unlike other cells in an organism, mature neurons cannot divide and usually have large expanses of dendritic and axonal cytoplasm. They consequently face particular hurdles in preventing cellular waste and misfolded proteins from accumulating over a lifetime without the aid of cell division to dilute these burdens. Young neurons achieve this task by efficient stress response and clearing systems supported by robust cellular signaling transductions. In comparison, the stereotypic neuronal connections in the elderly allow transformation and accumulation of specific proteins, such as A β , tau, α -syn and prion, easily within the nervous system. In respect to this, aged brain quite often is the organ affected most severely under conditions of NDs with altered activities of pathways in proteostasis (**Figure 1**). Although it is still uncertain whether the abnormal pathway activities implicate a primary cause or secondary consequence, the current chicken-and-egg debates concerning this issue, as outlined above, definitely will provide in-depth understandings of NDs, as well as a fruitful source of knowledge for therapeutics to treat these brain symptoms in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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