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Spreading of pathology in neurodegenerative diseases: a focus on human studies

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Abstract

The progression of many neurodegenerative diseases is thought to be driven by the template-directed misfolding, seeded aggregation and cell–cell transmission of characteristic disease-related proteins, leading to the sequential dissemination of pathological protein aggregates. Recent evidence strongly suggests that the anatomical connections made by neurons — in addition to the intrinsic characteristics of neurons, such as morphology and gene expression profile — determine whether they are vulnerable to degeneration in these disorders. Notably, this common pathogenic principle opens up opportunities for pursuing novel targets for therapeutic interventions for these neurodegenerative disorders. We review recent evidence that supports the notion of neuron–neuron protein propagation, with a focus on neuropathological and positron emission tomography imaging studies in humans.

Neurodegenerative diseases are a major cause of disability and premature death among older people worldwide[1–3]. Although these diseases, for which there are currently no disease-modifying therapies, show a great diversity of clinical phenotypes, they share a common pathological hallmark — the accumulation of characteristic proteins into insoluble aggregates in or among selectively vulnerable neurons and glial cells.

Aggregates of the phosphorylated microtubule-associated protein tau in neurofibrillary tangles and neuropil threads, together with deposits of amyloid-β (Aβ), are characteristic of sporadic Alzheimer disease (AD). Tau pathology alone also characterizes a subgroup of

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cases of frontotemporal lobar degeneration (FTLD), which is designated as FTLD-tau, as well as other rare tauopathies[4–7]. Moreover, neuronal accumulations of α-synuclein in Lewy bodies and Lewy neurites are the pathological signatures of sporadic Parkinson disease (PD) and PD with dementia, as well as of dementia with Lewy bodies[8]. Furthermore, almost all cases of amyotrophic lateral sclerosis (ALS) and a further subgroup of cases of FTLD (FTLD-TDP) are characterized by aggregates of TAR DNA-binding protein 43 (TDP43)[9].

The disease-related proteins are transformed from their normal conformation into fibrillar or multimeric species that function as seeds and templates to drive non-pathological protein counterparts to adopt a similar structural alteration (BOX 1). In a self-perpetuating process that has been likened to that observed in prion diseases[10], progressive seeded aggregation of conformationally altered proteins spreads to interconnected neurons and adjacent glial cells. Based on converging lines of evidence from human tissue, cell culture and animal model studies[11–13], the paradigm of pathological protein propagation in neurodegenerative diseases is now firmly established.

This Review links experimental evidence of the molecular mechanisms of protein propagation in AD (involving tau and Aβ), FTLD-tau (involving tau), PD (involving α-synuclein), and ALS and FTLD-TDP (both of which involve TDP43) to what is known from studies in humans regarding vulnerable types of neurons and glial cells and the pathways by which disease-protein pathology might spread through the CNS. We focus on studies of neuropathology but also underline the importance of novel protein-specific neuroimaging markers that offer the opportunity to validate the possibility of sequential spreading that has been implicated by human pathology studies. Finally, we discuss how the unifying pathological principle of protein propagation may offer new disease-modifying therapeutic approaches to treating neurodegenerative diseases.

Molecular mechanisms

In prion disease, an infectious protein replicates by recruiting and inducing pathological conformational changes in its normal counterpart, resulting in the aggregation of pathological prions[14–16]. Prions thus act as corruptive templates that induce a chain-reaction-like process of protein misfolding and progressive aggregation. Although such propagation mechanisms were long thought to be exclusively associated with prion diseases, recent studies have provided convincing evidence that a ‘prion-like’ self-propagating mechanism may apply to a wider range of proteins that are associated with neurodegenerative diseases, including misfolded Aβ, tau and α-synuclein, mutant huntingtin with polyglutamine repeats (which is characteristic of Huntington disease), mutant superoxide dismutase 1 (SOD1) and phosphorylated TDP43 (BOX 2; TABLE 1).

Initiation of protein pathology

To date, the mechanisms that trigger the initial conversion of normally soluble proteins into filamentous polymers remain unresolved (FIG. 1). In template-directed misfolding, a misfolded version of a protein interacts directly with the native protein and converts the latter into a misfolded replicate. For example, mutant SOD1 can induce the misfolding of native, wild-type dimeric SOD1 in human mesenchymal and neural cell lines[17], and

aggregates that contain both mutant and wild-type SOD1 have been found in individuals with familial ALS[18] and in mice that express both wild-type and mutant human SOD1 (REF. 19).

Seeded aggregation is the process whereby mis-folded proteins recruit and initiate template-directed misfolding of the native protein to form new aggregates. The first non-prion neurodegenerative disease protein that was shown to disseminate in this way was misfolded Aβ in marmosets; subsequently, seeded aggregation was also observed in mice overexpressing a mutant variant of the amyloid precursor protein, from which Aβ is derived[20–24]. Similarly, when brain extracts from mice expressing mutant human tau were injected into the hippocampus and the over-lying cerebral cortex of transgenic animals expressing wild-type human tau, tau pathology was seeded that went on to spread progressively[25]. Tauopathy was even observed in wild-type mice after seeding with exogenously prepared aggregates of tau, although there was a lower density of aggregates than in transgenic mice that expressed human mutant tau[26]. Furthermore, synthetic preformed tau fibrils induced tau aggregation in cultured cells and in transgenic mice that expressed human mutant tau[27,28]. Remarkably, recent studies have also demonstrated that tau, Aβ and α-synuclein exist in different conformational variants, or ‘strains’, that show different seeding and/or cross-seeding properties and different levels of neurotoxicity, and could thereby contribute to the tremendous heterogeneity of neurodegenerative diseases[29–31].

Early *in vivo* evidence to show that α-synuclein pathology can propagate originated from studies on individuals with PD who had received transplants of fetal mesencephalic dopaminergic neurons, which developed α-synuclein-positive Lewy bodies and showed signs of neuronal degeneration[32–36]. Such studies were followed by *in vitro* studies[37–39] and animal model experiments[37,38,40–47] that demonstrated seeded aggregation and transmission of α-synuclein. In contrast to the evidence for tau and α-synuclein transmission, evidence for the transmission of TDP43 is only beginning to emerge and to date is limited to *in vitro* studies[48,49].

Propagation

The mechanisms by which misfolded proteins can propagate from one neuron to the next (or, in the case of extracellular Aβ aggregates, from one region of the CNS to another) remain to be fully determined. Generally, for intracellular aggregates to propagate in this way, these pathogenic proteins need to be released from the originating neuron or glial cell and taken up by a neighbouring neuron or glial cell. Aβ (similar to prion protein) is naturally present in the extracellular space; however, tau, α-synuclein and TDP43 need to cross cell membrane barriers to propagate among cells. Although tau aggregates could be released into the extracellular space as fibrils[50], neuronal cell culture studies have shown that non-fibrillar (that is, monomeric or oligomeric) α-synuclein and tau could be secreted via exosomes[51–54]. Fluid-phase and receptor-mediated endocytosis have been implicated in the cellular uptake of mutant tau, mutant SOD1, and fibrils, oligomers and monomers of α-synuclein[39,55–59], and there is evidence that mutant huntingtin aggregates are capable of directly penetrating cell membranes without passage through endocytic compartments[60]. Neurodegenerative

disease proteins might be transferred among the bodies and processes of cells via several of these mechanisms simultaneously. Notably, this transfer may occur across synapses and thus correspond to intrinsic connectivities between cells[11].

Importantly, there is evidence to indicate that tau and α-synuclein can spread via neuronal connections (BOX 3). *In vitro* studies indicate that mutant and wild-type tau, fibrillar α-synuclein and wild-type TDP43 can be transported through the axon anterogradely as well as retrogradely[39,61–63] (and the transport of tau has recently been summarized in detail[64]). In transgenic mice expressing mutant human tau or mutant human α-synuclein, injection of tau or α-synuclein preformed fibrils, respectively, into the brain caused aggregates in anatomical structures that were relatively distant from the original injection sites, whereas no aggregates formed at the injection site[28,42], thus implying that neuronal connections are probably involved in disease protein propagation and disease spread. In addition, intramuscular injection of mutant α-synuclein in two mouse strains — one of which expressed mutant human α-synuclein and the other of which expressed wild-type human α-synuclein — caused intraneuronal α-synuclein pathology in the brain and the spinal cord[65].

Evidence from studies in humans

In this section, we review evidence from post-mortem and positron emission tomography (PET) studies of human tissue, and explain how these studies support the concept that neurodegenerative disease progression (in the disorders discussed here) probably reflects the cell–cell propagation of non-prion disease proteins.

Stereotypical patterns of pathology progression

Neuropathological studies have identified that stereotypical patterns of pathology occur in various neurodegenerative diseases over time, and that progression of these patterns is associated with increasing severity of the clinical phenotype, thus enabling the development of staging systems for these diseases. These studies noted that pathological lesions develop at different CNS sites sequentially, and that disease progression is associated with an increase in the size of aggregates and an increase in the number of cells showing pathological inclusions at these sites[66,67]. Such a stereotypical pathology pattern was first established for AD, in which tau aggregates are found first in the locus coeruleus of the pontine tegmentum[68] and then in the transentorhinal cortex of the anteromedial temporal lobe (FIG. 2a,b). Subsequently, lesions are found in other parts of the temporal lobe, including the entorhinal and hippocampal areas, with lesions next becoming detectable in the basal temporal lobe and the insular cortex, before finally developing in the neocortex[66,69]. Stereotypical patterns of tau pathology are not restricted to AD; tau lesions in chronic traumatic encephalopathy can originate close to perivascular spaces within the depths of cortical sulci[70] and become subsequently detectable in large regions of the neocortex and allocortex, diencephalon, basal ganglia, brainstem and spinal cord[7]. Thus, even though tau pathology characterizes both AD and chronic traumatic encephalopathy, the overall directions in which this pathology progresses in these diseases are different.

In contrast to the dissemination of tau, patterns of Aβ plaques in AD follow essentially the opposite direction: plaques are initially found in the cortex and then, at later disease stages,

spread to the brainstem[71] (FIG. 2c,d). Indeed, studies have shown that Aβ plaques are observed first in the neocortex and then in allocortical, diencephalic and basal ganglial structures before being found — in cases with the highest burden of pathology — in the brainstem and cerebellum[66,71,72]. Why these two major disease proteins of AD show such fundamentally different patterns is incompletely understood. Although tau and Aβ are likely to exert toxicity via separate mechanisms[73], observations from *in vitro* and *in vivo* models indicate that Aβ may drive the formation of tau pathology[74] and that tau may mediate Aβ toxicity[75–77]. Furthermore, these proteins could have synergistic deleterious effects on, for instance, mitochondrial function[78]. Future studies of possible interactions between these key players of AD pathology are needed to explain their divergent spreading patterns in AD[79].

Although tau pathology in AD is first found in caudal areas and then later in rostral regions, and Aβ plaque pathology is detected in increasingly caudal areas with disease progression, α-synuclein-containing Lewy bodies and Lewy neurites in PD, PD with dementia, and dementia with Lewy bodies are first detected in ventral areas and subsequently in more dorsal brain regions[69,71,80]. The earliest lesions in PD and in dementia with Lewy bodies can be detected in the olfactory bulb and anterior olfactory nucleus, as well as in the dorsal motor nucleus of the vagus nerve in the medulla oblongata. In PD and in dementia with Lewy bodies, with increasing burden of pathology, α-synuclein aggregate pathology is found in the pons and midbrain before being found in the basal forebrain and, ultimately, in the neocortex[80–85] (FIG. 2e,f). Thus, only in more advanced stages of PD does α-synuclein aggregation cause the loss of midbrain dopaminergic neurons in the pars compacta of the substantia nigra[80]. It is this specific cell loss that has been linked to the classic motor symptoms of PD, including rigidity, resting tremor and bradykinesia. The accumulation of α-synuclein aggregates in the anterior olfactory nucleus[86] and olfactory bulb is clinically reflected by hyposmia, which is frequently observed before the onset of motor symptoms in PD[87].

Intriguingly, in individuals with early PD, α-synuclein pathology has also been detected outside the CNS, in neurons of Auerbach's and Meissner's plex-uses of the enteric nervous system (ENS)[88,89], as well as in autonomic nerves of the submandibular gland[90]. This distribution of α-synuclein pathology could explain other early symptoms of PD, such as dysphagia and constipation[91], and has prompted speculation about the possible spread of early α-synuclein pathology between the closely connected ENS and the dorsal motor nucleus of the vagus nerve. However, it currently remains unclear whether it is the olfactory bulb, the ENS or the lower brainstem that is the earliest focus of α-synuclein pathology[92–94]. Indeed, the appearance of the initial pathology in these separate locations gave rise to the ‘dual-hit theory’ of PD[95].

Our group recently proposed four sequential stages of TDP43 pathology in ALS, whereby the protein pathology initially seems to spread rostrally and caudally from the motor neocortex and towards the spinal cord and brainstem, and then to frontal, parietal and, ultimately, anteromedial temporal lobes at later stages[96] (FIG. 2g,h). Moreover, other studies show stereotypical patterns of TDP43 pathology that are suggestive of sequential spreading in other neurodegenerative diseases. Indeed, TDP43 aggregates have frequently been observed in addition to tau pathology and Aβ plaques in AD[97–102]. In such cases,

TDP43 aggregates have been reported to first appear in the amygdala and, with increasing burden of pathology, to be subsequently detected in the entorhinal and hippocampal areas, the occipitotemporal and inferior temporal cortical areas and, finally, the frontal cortex and basal ganglia[103]. Similarly, in FTLD-TDP presenting as behavioural variant FTD, a stereotypical distribution pattern suggestive of a sequential spreading of TDP43 aggregates along a fronto-occipital gradient was observed, with the first lesions developing in orbitofrontal areas and the amygdala, and other lesions appearing later in premotor, primary motor, parietal and, finally, occipital areas of the cortex[104]. In chronic traumatic encephalopathy, TDP43 pathology has been observed among widespread cortical regions (as in FTLD-TDP), as well as in the spinal cord, although no clear regional spreading pattern has been described[7]. It is therefore possible that TDP43 pathology spreads along different pathways in different neurodegenerative diseases.

Vulnerable types of neurons

Human tissue studies and animal model experiments indicate that the spatiotemporal patterns of neurodegenerative disease protein pathology are highly selective. Only a small number of the many neuronal types are prone to developing the abnormal aggregations, with the remainder — often directly in the vicinity of the affected neurons — remaining functionally and morphologically normal. For instance, α-synuclein pathology in early multiple system atrophy is observed in neurons of the inferior olive in the medulla oblongata[105], whereas the dorsal motor nuclei of the vagus nerve and the hypoglossal nerves remain virtually free of α-synuclein aggregates (except in patients with severe overall pathology). The reasons underlying this selective vulnerability of distinct neuronal populations are unknown. Neuronal vulnerability could be determined by inherent biochemical and structural characteristics that would render neurons more prone to developing aggregates, and/or by the location and connectivity of these cells. Glial cells, particularly oligodendrocytes, exhibit α-synuclein inclusions early in multiple system atrophy[105]; however, the mechanisms of possible spread between glial cells and neurons remain largely unknown (BOX 4).

Several studies have noted that neurons affected by the aggregation of α-synuclein, tau or TDP43 have specific anatomical characteristics. In PD, the neurons found to be particularly vulnerable to α-synuclein aggregation belong to the class of projection neurons[80]. Specifically, projection neurons with axons that were very long exhibited a pronounced tendency to develop α-synuclein inclusions. By contrast, projection neurons with short axons (such as the pyramidal cells of neo cortical layers II and IV, the neurons of the presubicular parvocellular layer of the hippocampus and local-circuit neurons with short axons) were generally resistant to α-synuclein aggregation[80]. Furthermore, projection neurons with long and thin axons that were only sparsely myelinated or unmyelinated were vulnerable to α-synuclein aggregates, whereas neurons with long, but thickly myelinated axons with large diameters were resistant to the formation of such aggregates[80,106].

It has been suggested that projection neurons with sparsely myelinated axons would require prodigious energy expenditure to maintain axonal function and transport[107], and that such high energy demands would result in continuously high levels of oxidative stress that could

increase neuron vulnerability to α-synuclein aggregation in PD[108–111]. In the final stages of PD (when α-synuclein aggregates can be found in the neocortex), the cortical areas that myelinate ontogenetically last and therefore have thinly myelinated axons develop severe α-synuclein pathology, whereas thickly myelinated axons in primary cortices are comparatively impervious to α-synuclein aggregation[112]. Intriguingly, a highly similar pattern can be noted in AD: the first tau aggregates appear in the sparsely myelinated temporal mesocortex, whereas heavily myelinated primary cortical fields are the last to exhibit such pathology[113].

Moreover, motor neurons are very large with very long axons that require high levels of mitochondrial activity compared with other neurons, and this could make them more vulnerable in ALS[114]. Importantly, however, neuronal susceptibility to TDP43 aggregation in ALS is not confined to motor neurons but also includes non-motor neurons such as the pyramidal layer II or III cells of large (non-motor) areas of the neocortex, especially in frontal areas[96]. By contrast, some motor neurons — for example, the motor neurons of the cranial nerves III, IV and VI that innervate the extrinsic eye muscles — are not (or are only at very late stages of disease) affected by TDP43 lesions[96].

Routes and pathways

Imaging studies of patients with FTD or AD suggest that the differential vulnerability of brain regions to neurodegenerative changes is correlated with the strength of neuronal connections among the involved areas[115,116]. For example, brain regions with intense neuronal connectivity — as determined by functional MRI — correlated with foci of brain atrophy in patients with behavioural variant FTD or other neurodegenerative diseases[115]. Neuropathology studies of ALS, PD and AD also provide evidence to suggest that neurodegenerative protein pathologies may spread via neuronal connections.

In ALS, the vulnerability of subcortical neurons to developing TDP43 aggregates seems to be determined by the presence of direct cortical connections from already affected areas. Post-mortem analysis of ALS brain tissue revealed that areas that receive direct projections from the cortex develop TDP43 inclusions once the corresponding area of cortex is affected[96]. By contrast, neurons that receive no relevant cortical input — such as the motor nuclei of the extrinsic eye muscles — remain resistant to TDP43 lesions until late in the disease[96].

In PD, the detection of α-synuclein pathology in the vagal dorsal motor nucleus in cases with early disease is intriguing because this nucleus is a synaptic relay station of the parasympathetic nervous system, which includes the ENS, and Lewy body and Lewy neurite pathologies have been repeatedly observed in neurons of the ENS[89,117–119]. The mechanisms that trigger misfolding of α-synuclein in the ENS remain unresolved, although local inflammation, oxidative stress and the crossing of external pathogens through the mucosal barrier to the ENS are all proposed triggers[112,119].

In individuals with AD, or PD and concomitant AD, glial tau pathology is rare, but all of the brain regions and neuronal types that do exhibit tau pathology are anatomically interconnected. This pattern indicates that physical contacts, axonal transport and/or

transynaptic transmission between involved regions could play a key role in the pathogenesis of AD[120–122]. The locus coeruleus — the initial site of tau pathology in AD — is anatomically distant from the cortical transentorhinal region, the second site in which pretangles (and subsequently, neurofibrillary tangles) develop in AD. Tau aggregates are at first confined to isolated cortical pyramidal cells that are located chiefly in the transentorhinal region[11,25,120,123,124]; however, axonal projections from the coeruleus project to this region, suggesting that these projections may mediate the propagation of tau pathology.

Some nuclei within the brainstem (including the locus coeruleus), midbrain, basal forebrain and hypothalamus send long and extensively ramifying projections to the olfactory bulb, many subcortical nuclei, the entire cerebral cortex, the cerebellum and the spinal cord. Such nuclei, in contrast to specific thalamic nuclei (the neurons of which project and relay data to specific cortical regions), belong to a functionally unified group called the non-thalamic nuclei with diffuse cortical projections, and these projections are assumed to have a more generalized effect on cortical regions[125]. Even at early stages of AD, all of these diffusely projecting non-thalamic nuclei display some degree of tau pathology[126–128].

Phylogenetic influences may also be partially responsible for the spread of tau pathology from the locus coeruleus to cortical neurons in the transentorhinal region in AD. The transentorhinal region is an interface between the basal temporal neocortex (which develops late, both phylogenetically and ontogenetically speaking) and the portions of the entorhinal region that are close to the neocortex. Among primates, humans have the largest transentorhinal region and the transentorhinal region that shows the highest degree of laminar differentiation[129]. The most evolutionarily recent portions of the locus coeruleus project to and synapse in the transentorhinal region. The fact that this connection is evolutionarily recent may partially explain why neurons from these two areas develop the earliest AD-associated tau lesions within the CNS[68,122,130].

PET studies

Human tissue pathology-staging studies are limited by the relative lack of early (that is, prodromal) cases, because most patients die as a consequence of the neurodegenerative disease. Furthermore, these studies are limited by the fact that the resulting neuropathological data are by definition cross-sectional, and can propose a sequential order of involvement only by correlating findings with clinical phenotype and disease duration. Although experimental evidence from animal studies suggests a similar spreading in humans is most likely, this cannot be proven in any given individual autopsy case.

To validate the sequential involvement of different CNS regions proposed by human autopsy studies, imaging biomarkers specific to the different disease proteins are necessary because they should enable the *in vivo* detection and monitoring of any spreading of protein pathology in longitudinal studies. Currently, the most promising markers are PET ligands. Such markers have been developed to visualize Aβ pathology in individuals with AD, and PET ligands for pathological tau are also emerging[131,132].

The PET tracer that is most widely used for imaging Aβ is ^{11}C-6-OH-BTA-1, which is also known as ^{11}C-Pittsburgh compound B (^{11}C-PiB), which was developed by Klunk and colleagues[133]. ^{11}C-PiB PET-imaging trials in humans have shown that tracer uptake in the neocortical brain regions of patients with AD is substantially greater than in those of individuals without dementia[133]. Moreover, trial data have shown that tracer uptake is predictive of conversion of mild cognitive impairment to AD[134] and correlates with Aβ deposition (as determined post-mortem)[133,135]. Individuals carrying rare autosomal-dominant AD mutations showed elevated ^{11}C-PiB levels in nearly every cortical region and also in subcortical grey-matter structures 15 years before the estimated age of dementia onset[136]. In addition to ^{11}C-PiB, several studies have used the ^{18}F-labelled Aβ-targeting tracers flutemetamol[137], florbetapir[138] and florbetaben[139]. Flutemetamol showed good discrimination between AD and controls[137], and florbetapir showed close correlation of ^{18}F uptake in PET with post-mortem Aβ pathology[138]. Furthermore, florbetaben showed a higher tracer uptake in individuals with AD than in age-matched normal controls, especially in neocortical regions (particularly the posterior cingulate gyrus)[139]. Moreover, there was a lower uptake of an Aβ-specific PET ligand within the hippocampus than in neocortical brain areas, and this may closely reflect the more prominent Aβ pathology in neocortical areas than in allocortical areas, as reported in post-mortem neuropathology studies[71]. A caveat, however, is that Aβ-specific PET tracers may bind to fibrillar rather than to early-stage diffuse Aβ plaques; therefore, the earliest deposits of Aβ may not be visualized by these imaging ligands. In addition, ^{11}C-PiB and the ^{18}F-labelled PET tracers bind with greater affinity to β-sheet (fibrillar) Aβ than to diffuse plaques[140].

Several PET markers that are highly specific to neurofibrillary tangles and other types of tau pathology (including glial tau pathology) have recently been developed, such as the ^{18}F-labelled tracers ^{18}F-THK5105 (REF. 184), ^{18}F-THK523 (REF. 132), ^{18}F-T807 (REF. 141), ^{18}F-T808 (REF. 142) and the ^{11}C-labelled tracer ^{11}C-PBB3 (REF. 143). *In vitro* studies in AD brain homogenates have shown that THK5105 and T808 have an affinity for tau fibrils that is 25–27-fold higher than that for Aβ fibrils[144]. In a group of eight individuals with late-stage AD, the ^{18}F-THK5105 PET tracer was substantially enriched in the medial and inferior temporal lobe (as compared with cognitively normal age-matched controls)[145], thus reflecting the early effects of tau neurofibrillary tangle pathology on these areas in AD[69]. In addition, ^{18}F-THK5105 retention was strongly associated with decreases in cognitive parameters and with reduced hippocampal and whole-brain grey-matter volumes. All of these associations are consistent with findings from previous post-mortem studies that showed marked correlations between neurofibrillary tangle density and dementia severity or neuronal loss[69].

A notable feature of the tau tracers is that they have different sensitivities to different types of tau lesions depending on the underlying disease. Tau exists in six isoforms: three three-repeat (3R) isoforms and three four-repeat (4R) isoforms. Thus, tau deposits can contain 3R tau, 4R tau or a mixture of 3R and 4R tau iso-forms, depending on the specific disease[6]. The ^{18}F-tracer THK523 exclusively labels neurofibrillary tangles (which contain a mixture of 3R and 4R isoforms) that are present in AD, but not the 3R tau lesions that occur in Pick disease or the 4R tau lesions that occur in corticobasal degeneration and progressive supra-

nuclear palsy, whereas ^{11}C-PBB3 seems to detect all of these tau lesions[143]. Thus, these tracers possess different clinical applications, either as general markers of AD and related tauopathies (for instance, in the case of ^{11}C-PBB3) or for the differential diagnosis to distinguish between tauopathies (for example, ^{18}F-THK523).

A current limitation of PET studies is their relatively low spatial resolution, which makes it difficult to adequately detect neurofibrillary tangles within subcortical regions and small anatomical structures such as the locus coeruleus (which is where, according to neuropathological staging models, tau pathology first occurs in AD[69]). Thus, compared with autopsy staging studies that can focus on individual lesions and involved cells, PET studies can only provide an assessment of regional effects. In addition, the majority of PET studies have been cross-sectional; to show a sequential spread of pathology, additional longitudinal studies are necessary that should also compare their findings to those from post-mortem pathology analyses. Finally, there are still no specific neuroimaging markers for synucleinopathies or TDP43 proteinopathies, although the development of such ligands is the focus of intense ongoing research. Indeed, for these latter diseases, we are currently limited to indirect approaches that monitor the neuronal and axonal loss that is associated with potentially spreading pathology. Given the close relationship between the presence of protein deposits and neuronal loss (as seen in pathology studies of tau in AD or TDP43 in ALS and in FTLD-TDP[69,96,104]) in certain regions, serial analyses of changes in brain connectivity[115] and of progressive damage to fibre tracts could help to delineate spreading pathways in proteinopathies for which no specific *in vivo* marker yet exists. For example, we demonstrated that ALS-induced axonal loss in white-matter tracts — as measured by diffusion tensor imaging — affected the same regions as seen in our staging study of TDP43 pathology[146], and we are currently implementing this diffusion tensor imaging-based approach in a prospective study. However, prospective studies that implement serial fibre-tract and connectivity analyses are still rare and have been limited to AD[147]. Moreover, these prospective studies have mostly included small numbers of individuals and have not compared their findings with data from post-mortem analysis of protein pathology.

Therapeutic implications

To date, no disease-modifying therapy exists for the neurodegenerative diseases discussed here, and neuroprotective and anti-inflammatory therapies have largely proved unsatisfactory. Template-directed replication and subsequent cell–cell transmission of pathology-associated proteins provides a common molecular pathway that could be targeted by novel therapeutic strategies with the aim of disrupting or delaying propagation.

As template-directed misfolding is likely to be a very early event in the pathological cascade of the non-prion neurodegenerative diseases discussed here, one therapeutic approach could be to stabilize the wild-type proteins in their normal conformation, ideally rendering them resistant to template-directed conformational change while not interfering with their normal cellular function[13]. This approach has already been applied with some success to the treatment of transthyretin amyloidosis[148]. In this disease, the transthyretin tetramer must first dissociate into monomers to form amyloid. Ligands that stabilize the tetramer can prevent amyloidosis, and a drug with that effect (tafamidis) has been approved by the

European Medicines Agency for the treatment of a familial form of transthyretin amyloidosis[148].

Moreover, agents that interfere with the release or uptake of neurodegenerative disease proteins could prevent transmission of pathology to neighbouring neurons. For example, specific antibodies could capture protein seeds in the extracellular space as they are 'in transit' between neurons[149], or target receptors or other cellular proteins needed for the uptake or release of pathogenic proteins[10,13,17,40,50,150,151]. However, such immunological interventions in humans would face the formidable obstacles of the blood–brain barrier and the blood–cerebrospinal fluid barrier, both of which limit the passage of extrathecally administered antibodies into the CNS[152]. Similarly, interfering with protein transmission by nonspecifically enhancing cell membrane stability, inhibiting endocytosis or inhibiting exosomal protein secretion would probably interfere with the homeostasis of other cellular proteins and thus entail unacceptable adverse effects. However, these therapeutic strategies are worthy of further exploration.

Open questions and future directions

Although the propagation of disease proteins offers a unifying pathophysiological concept of neurodegenerative diseases, several key issues need to be resolved. The specific molecular mechanisms that trigger the initial conversion of normally soluble proteins into filamentous or aggregated polymers remain unknown. Understanding the initial event or events in the protein-misfolding cascade would be valuable for identifying therapeutic targets that could be modulated before potentially irreversible spreading of protein pathology and neuron loss.

Furthermore, it will be crucial to determine the specific biochemical characteristics of protein species that are transmissible. For instance, it remains to be determined whether pre-fibrillar protein species, such as oligomers, are more detrimental to cells than are fibrillar forms[153]. Many mechanistic aspects of cell–cell transmission require clarification. Although the uptake and release of pathological proteins via different types of endocytosis and exocytosis processes can explain pathological protein transmission in cell culture models, they can only partially account for the spreading of disease proteins over considerable anatomical distances as observed in animal models and human tissue studies[62,154]. An understanding of the pathways and directionality of spreading would be facilitated by the definition of primary (early) foci of protein pathology in the human disease. However, for some diseases, such as PD, the initial focus is still being discussed[95], and for others, such as ALS, it has not been determined[96]. This is attributable partly to the limitations of neuropatho-logical studies, which analyse selected and often only small blocks of tissue (obviously, with the aim of preserving invaluable human material) that do not allow detailed analysis of pathology in large regions or across different regions of the CNS. Thus, comprehensive pathology studies performed on entire cortical regions or entire hemispheres, the brainstem and the full length of the spinal cord may be necessary to more reliably define the early foci of protein pathology.

Finally, many neurodegenerative diseases are likely to be non-cell autonomous, with an important part played by astroglia, oligodendroglia and microglia. For example, in multiple

system atrophy, α-synuclein pathology chiefly presents as cytoplasmic inclusions in oligodendrocytes (although it is also observed to a much lesser extent in neurons)[105]. The relevance and extent of glial contributions to the transmission of protein pathology in neurodegenerative diseases remain unclear.

Considerable effort will be needed to resolve each of these problems and to make progress towards effective therapies for neurodegenerative diseases. Indeed, any progress in resolving the key issues outlined in this Review is likely to advance our understanding of each of these diseases substantially, and future strategies that target the transmission of any of these pathological proteins will most probably constitute a fundamental step forward in treating neurodegenerative diseases.

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Box 1 | Structural characteristics of neurodegenerative disease proteins

The misfolded conformations of amyloid-β, tau, α-synuclein, huntingtin and superoxide dismutase 1 are characterized by bundles of twisted, non-branching filaments that are composed of β-sheets that in turn consist of β-strands[13].

These filaments, known as amyloids, are defined by specific X-ray diffraction patterns, as well as by chemical stains such as Congo red, Thioflavin-S and Thioflavin-T[155]. TAR DNA-binding protein 43 (TDP43) aggregates are less amyloid-like than the pathological proteins mentioned above: TDP43 inclusions mainly contain TDP43-immunoreactive, amorphous disordered aggregates, in which occasional 10–20 nm-diameter straight filaments are found[156]. However, a recent study in human tissue examining a small set of inclusions also observed skein-like inclusions composed of amyloid-like filamentous TDP43, indicating that a certain proportion of TDP43 inclusions are amyloid-like[157]. Furthermore, human-derived carboxy-terminal fragments of both wild-type and mutant TDP43 formed Thioflavin-T-reactive fibrils in culture[158]. In addition, the C-terminal region of human TDP43 has a prion-like domain[159], and this was suggested to be required for amyloid-like fibril formation. Importantly, several studies suggest that pre-fibrillar species of neurodegenerative disease proteins may be more detrimental than fibrillar species[153]. For example, amyloid-β oligomers were suggested to induce synaptic dysfunction by interfering with signalling pathways downstream of certain NMDA or AMPA receptors at synaptic plasma membranes[153].

Sequential spreading

The staged spatial dissemination of intracellular or extracellular protein aggregate pathology in neurodegenerative diseases.

Skein-like inclusions

Elongated filamentous intraneuronal inclusions of neurodegenerative disease proteins.

Cross-seeding

A process whereby seeds of one pathological protein cause the aggregation of another protein.

Exosomes

Cell-derived vesicles that are released from the plasma membrane and could thus constitute a mechanism of disease-protein release in neurodegenerative diseases.

Box 2 | Neurodegenerative disease proteins: prions or not?

Prions arise from an abnormal version of the cell membrane prion protein PrP^{C}, called PrP^{Sc}, which misfolds and may aggregate into plaques. The name was coined by Prusiner in 1982 when he suggested that the scrapie agent be designated a “proteinacious infectious particle” or “prion” (REF. 14). Prions isolated from different sources (such as sheep, deer and cattle) target different brain areas, induce unique symptoms and have varied incubation times, indicating that prions exist in different conformational variants — so-called strains[160,161]. Prions are by definition infectious, and have caused epidemics[162]. Accordingly, infectivity is one of the essential components of the definition of prions. The infectious transmission of prions between species is not trivial and thus justifies categorizing infectious prion diseases as zoonoses. This was dramatically evidenced by the spread of bovine spongiform encephalopathy (which killed almost 200,000 cattle) to humans in the form of Creutzfeldt–Jakob disease, which caused more than 200 human deaths[162].

Prionoids (also called ‘prion-like proteins’ to emphasize their similarities to prions, or ‘non-prion proteins’ to underline differences) is a term coined by Aguzzi[10] to refer to disease proteins that seem to spread from cell to cell, possibly underlying the progression of non-prion neurodegenerative diseases such as Alzheimer disease and Parkinson disease[11,13]. Evidence from animal model studies and cell culture experiments indicates that misfolding, cell–cell propagation and spreading of prionoids within regions probably occurs in non-prion neurodegenerative diseases, and there is increasing evidence to indicate that these disease proteins can also exist as different strains[29–31]. However, although misfolded variants of these proteins from one diseased animal can, when injected at high concentrations into the brains of unaffected animals, spread in the recipient, they cannot be termed prions because they are neither infectious nor zoonoses in the way prion diseases are[161,163]. There are no epidemiological data to support an infectious spread of tauopathies and synucleinopathies through blood transfusions, organ transplants or other means. Indeed, we found no evidence of inter-individual transmission of any of these diseases in a cohort of more than 7,000 middle-aged and older individuals who, as children, had been treated with daily systemic injections of human growth hormone extracted from post-mortem human pituitaries, whereas 24 cases of prion PrP^{Sc} disease occurred[163].

An even more notable difference between prions and other transmissible neurodegenerative disease proteins, again with respect to infectivity, comes from data on the very high prevalence of readily transmissible or infectious prion diseases in sheep, cattle, moose, elk and other animals for which there is just no counterpart for tauopathies or synucleinopathies. Nothing comparable to the epidemic of, for instance, bovine spongiform encephalopathy has occurred for tauopathies or synucleinopathies in livestock, and nor is anything likely to occur, because there is no reservoir of infectious human proteinopathies in livestock or other mammals. These clinical and epidemiological differences are important for human medicine, and suggest that prion diseases and neurodegenerative diseases caused by non-prion neurodegenerative disease proteins or prionoids should not be grouped together. In agreement with Aguzzi[161], and

to avoid unfounded public health concerns about whether Alzheimer disease, Parkinson disease and other non-prion neurodegenerative diseases may be infectious, we do not recommend referring to these other disease proteins as prions. Instead, we think it is preferable to refer to these non-prion neurodegenerative disease proteins by their long-accepted traditional names — pathological amyloid-β, tau and α-synuclein — rather than as amyloid-β, tau and α-synuclein prionoids.

Chronic traumatic

encephalopathy

A form of neurodegeneration that occurs in individuals who have sustained multiple concussions or injuries to the brain.

Rigidity

Stiffness and resistance to limb movement due to increased muscle tone. It is a key clinical symptom of Parkinson disease and can be uniform (known as lead-pipe rigidity) or ratchety (known as cogwheel rigidity).

Hyposmia

A reduced ability to smell and detect odours. This deficit can be due to any of a wide range of causes, including neurodegenerative diseases such as Parkinson disease or Alzheimer disease.

Dual-hit theory

The proposal that α-synuclein pathology in Parkinson disease starts in two different locations: the lower brainstem (dorsal motor nucleus of the vagus nerve) and the olfactory bulb.

Box 3 | Evidence from animal model studies of spreading via neuronal connections

If the vulnerability of neurons to protein pathology were determined solely by inherent neuronal characteristics, one would expect the same types of neurons to be affected, independently of the location of the initial focus. However, animal models in which the initial focus of pathology is varied have shown that depending on where preformed fibrils (PFFs) are injected, different neuronal populations are sequentially affected[25,28,42,44,124], implying that neuronal connectivity could be an important clue to understanding neuronal vulnerability to protein aggregation in neurodegenerative diseases. For example, injections of human tau PFFs into the striatum of PS19 human mutant tau transgenic mice induced tau aggregation in regions interconnected with the striatum, including the substantia nigra and thalamus, whereas after PFFs were injected into the hippocampus, aggregates first spread to the entorhinal cortex and contralateral hippocampus[28].

Furthermore, as inclusion pathology can spread to anatomically distant structures while neurons closer to the injection site are spared, the transmission of protein pathology in these diseases might not simply occur by diffusion among neighbouring cells but rather occur via axonal connections[25,28,42,124]. A recent study in mice expressing human mutant tau indicated that tau seeded aggregates, similar to prions and amyloid-β aggregates, can reach the CNS from the periphery[164]. Recently, the hypothesis that intestinal α-synuclein spreads to the CNS via postganglionic enteric neurons and the vagus nerve was strengthened by the observation that human Parkinson disease brain lysates injected into the intestinal wall of wild-type Sprague–Dawley rats cause α-synuclein pathology in the dorsal motor nucleus of the vagus after ~144 hours[165]. Although mechanisms such as glial cell–glial cell or glial cell–neuron spreading, migration through interstitial fluids and/or cerebrospinal fluid, or blood-cell-mediated transport of tau amyloid pathology[166] cannot be eliminated, the patterns of pathology in animal models and human tissue studies make the idea of neuronal connection-mediated propagation particularly attractive, at least for pathological tau and α-synuclein[154].

Pretangles

Early intracellular aggregates of hyperphosphorylated tau protein that develop into filamentous neurofibrillary tangles.

Box 4 | Do glia contribute to protein propagation?

There is now abundant evidence to support a prominent role of astrocytes, microglia and oligodendrocytes in the initiation and progression of different neurodegenerative diseases (recently summarized in REFS 167–170). By contrast, little is known about how glia could specifically contribute to protein propagation.

Some initial studies on the role of glia in protein propagation exist for synucleinopathies. Here, both monomeric and aggregated α-synuclein released from neuronal cells were shown to be endocytosed by astrocytes in a co-culture of primary astrocytes with a neuronal cell line overexpressing α-synuclein[171]. This neuron-to-astroglia transfer of α-synuclein led to changes in the expression of genes related to neuroinflammatory response[171]. The glial accumulation of misfolded α-synuclein through transmission of the neuronal protein was also demonstrated in transgenic mice expressing human α-synuclein[171]. These results indicate that astroglial α-synuclein pathology observed in Parkinson disease and dementia with Lewy bodies could be caused by a direct transmission of neuronal α-synuclein to astroglia, and could result in inflammatory responses, including microglial activation[172]. Suppression of this subsequent microglial activation extended mouse survival.

To replicate the prominent pathological effects on oligodendroglia in multiple system atrophy, several transgenic mouse models that express human wild-type α-synuclein under the control of oligodendroglia-specific promoters have been developed. These models reproduce the accumulation of α-synuclein in glial cytoplasmic inclusions and these mice exhibit various levels of motor impairment[173,174]. Indeed, Yazawa *et al.*[174] described α-synuclein pathology in axons of transgenic mice overexpressing wild-type human α-synuclein specifically in oligodendrocytes that at the time was difficult to explain, but that in retrospect may have signified the transfer of α-synuclein pathology from oligodendrocytes to axons.

In addition, oligodendrocytes in cell culture were shown to internalize α-synuclein monomers, oligomers and fibrils. Furthermore, in the brains of rats that overexpressed human α-synuclein, a transfer of human α-synuclein to rat oligodendroglia that had been grafted into the striatum was observed[175]. These findings support the notion of neuron-to-oligodendrocyte transfer of α-synuclein, a mechanism that could have a crucial role in the propagation of α-synuclein pathology in multiple system atrophy.

For tauopathies, Clavaguera *et al.*[26] showed the induction of tau pathology in oligodendrocytes and astrocytes following injection of progressive supranuclear palsy, corticobasal degeneration and argyrophilic grain disease brain lysates into the brains of mice expressing wild-type tau. This demonstrates that tau pathology can spread to these cells, but does not explain the potential role of glia in tau propagation.

Finally, with regard to amyloid-β, both in cases of Alzheimer disease and transgenic mice, the expression of β-secretase 1 and amyloid-β were not restricted to neurons but to also occur in astroglia[176], indicating that astrocytes may contribute to the generation of amyloid-β aggregates *in vivo*.

Thus, although the current evidence from cell culture studies and animal models is limited, it is plausible that pathological aggregates may be induced not only in neurons, but also (to a variable extent) in glial cells, and that pathological disease proteins may be transferred between neurons and glia. Therefore, future studies that more specifically examine the role of glial cells in protein propagation are necessary.

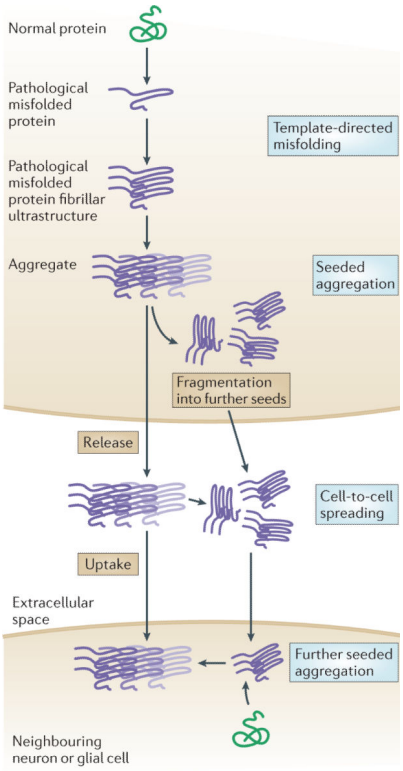

Figure 1. Hypothetical molecular mechanisms of prion-like disease protein transmission in neurodegenerative diseases

In template-directed misfolding, the deposited pathological disease proteins are transformed from their normal conformation, via intermediates, into fibrillar species. These species have the properties of amyloid (for instance, a fibrillar ultrastructure that consists of sheets of β-strands) and serve as templates to drive normal physiological versions of the protein to adopt similar structural alterations[13]. In a self-perpetuating process, the progressive seeded aggregation of conformationally changed proteins results in intracellular aggregates that fragment into ‘daughter seeds’. Finally, in cell–cell transmission, pathological proteins spread to anatomically interconnected neurons and adjacent glial cells via an autocatalytic chain-reaction-like process[11].

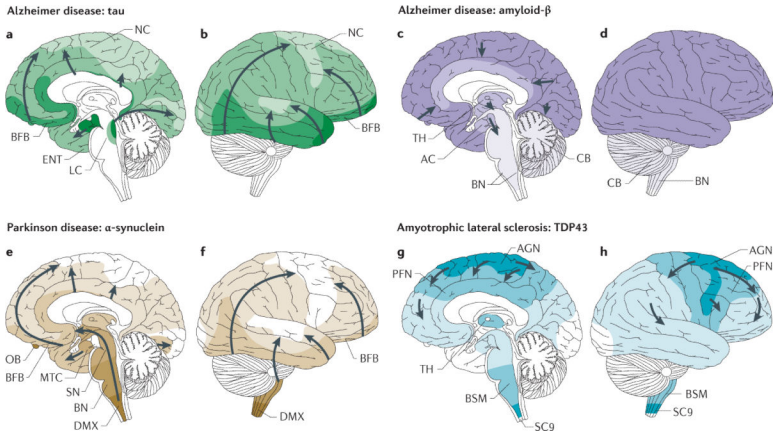

Figure 2. Sequential topographical dissemination of non-prion proteins in neurodegenerative diseases

In all panels, the pathology is first detected in areas delineated by darker colours and subsequently in regions shown in lighter colours. **a**, **b** | In Alzheimer disease, tau aggregates develop in the locus coeruleus (LC), then in the transentorhinal and entorhinal regions and subsequently in the hippocampal formation and in broad areas of the neocortex (NC)[69]. **c**, **d** | In contrast to tau pathology, amyloid-β deposits in Alzheimer disease are first observed in the NC and are then detected in allocortical, diencephalic and basal ganglia structures (in a caudal direction) and in the brainstem, and occasionally in the cerebellum (CB)[71]. **e**, **f** | The progression of α-synuclein-immunoreactive Lewy body and Lewy body and neurite pathology in Parkinson disease follows an ascending pattern from the brainstem to the telencephalon[80]. The earliest lesions can be detected in the olfactory bulb (OB), as well as in the dorsal motor nucleus of the vagus nerve (DMX) in the medulla oblongata. At later stages, the α-synuclein aggregate pathology is found more rostrally through the brainstem via the pons and midbrain, in the basal forebrain and, ultimately, in the NC. **g**, **h** | In amyotrophic lateral sclerosis cases with a low burden of TAR DNA-binding protein 43 (TDP43) pathology, TDP43 inclusions are seen in the agranular motor cortex (AGN), in the brainstem motor nuclei of cranial nerves XII–X, VII and V, and in α-motor neurons in the spinal cord. Later stages of disease are characterized by the presence of TDP43 pathology in the prefrontal neocortex (PFN), brainstem reticular formation, precerebellar nuclei, pontine grey and the red nucleus. Subsequently, prefrontal and postcentral neocortices, as well as striatal neurons, are affected by pathological TDP43, before the pathology is found in anteromedial portions of the temporal lobe, including the hippocampus[96]. AC, allocortex; BFB, basal forebrain; BN, brainstem nuclei; BSM, brainstem somatomotor nuclei; ENT, entorhinal cortex; MTC, mesiotemporal cortex; SC9, spinal cord grey-matter lamina IX; SN, substantia nigra; TH, thalamus. Part **f** reprinted from *Neurobiology of Aging*, 24, Braak, H. *et al.* Staging of brain pathology related to sporadic Parkinson's disease, 197–211, © 2003, with permission from Elsevier.

Table 1

Summary of evidence supporting the propagation and transmission of non-prion neurodegenerative disease proteins

Pathogenic protein	Associated diseases in humans	Main localization of protein		Studies in human tissue supporting sequential spread	References supporting neuron-neuron transmission	
		Wild-type	Pathological		Cell culture	Animal models
α-synuclein	PD, DLB and MSA	Presynaptic	Cytoplasmic	PD[80] and MSA[105]	39,40,62,177 178	41–44
Amyloid-β	AD	Transmembrane (APP)	Extracellular	AD[66],[71]	179	23,31,180–182
Mutant huntingtin	HD	Nuclear	Nuclear	–	60	–
Mutant superoxide dismutase 1	ALS	Cytoplasmic	Cytoplasmic	–	56	–
RNA-binding protein FUS	ALS and FTLD-FUS	Nuclear	Cytoplasmic	–	–	–
Tau	AD, FTLD-tau (including PiD, PSP and CBD) and CTE	Cytoplasmic	Cytoplasmic	AD[66] and CTE[7]	50,55	25,183
TDP43	ALS and FTLD-TDP	Nuclear	Cytoplasmic	ALS[96], FTLD-TDP[104] and AD[103]*	48,49	–

AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein (a transmembrane precursor protein whose proteolysis generates amyloid-P); CBD, corticobasal degeneration; CTE, chronic traumatic encephalopathy; DLB, dementia with Lewy bodies; FTLD, frontotemporal lobar degeneration; HD, Huntington disease; MSA, multiple system atrophy; PD, Parkinson disease; PiD, Pick disease; PSP, progressive supranuclear palsy; TDP43, TAR DNA-binding protein 43.

*Although AD is primarily characterized by tau and amyloid-P pathology, TDP43 pathology is observed in up to 57% of cases.