Prion-like Properties of Tau Protein: The Importance of Extracellular Tau as a Therapeutic Target

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Work over the past 4 years indicates that multiple proteins associated with neurodegenerative diseases, especially Tau and α-synuclein, can propagate aggregates between cells in a prion-like manner. This means that once an aggregate is formed it can escape the cell of origin, contact a connected cell, enter the cell, and induce further aggregation via templated conformational change. The prion model predicts a key role for extracellular protein aggregates in mediating progression of disease. This suggests new therapeutic approaches based on blocking neuronal uptake of protein aggregates and promoting their clearance. This will likely include therapeutic antibodies or small molecules, both of which can be developed and optimized in vitro prior to preclinical studies.

Neurodegenerative diseases account for an enormous human and financial cost to our society, estimated in excess of $200 billion annually (1). Despite decades of study, there is no disease-modifying therapy. Virtually all neurodegenerative diseases are associated with the accumulation of fibrillar protein aggregates, and all are relentlessly progressive. There is now abundant evidence for an association of neuronal networks with patterns of spread through the brain (2–4). Studies of relatively rare, dominantly inherited neurodegenerative diseases have indicated that proteins that accumulate in sporadic forms of disease, such as prion protein, Tau, α-synuclein, and TDP-43, also cause pathology in the setting of destabilizing point mutations (5–8). This provides a strong indication that protein aggregation is itself a proximal cause of disease and is not simply an epiphenomenon. Indeed, protein aggregation is the most unifying pathological feature of adult onset neurodegenerative disorders. The proximal initiators of protein aggregation likely vary among different proteins and cell types, whereas the accumulation of misfolded species in general appears to be linked to the age-dependent breakdown of cellular quality control pathways (9, 10). It is not understood, however, why neurodegenerative diseases are relentlessly progressive or why they involve neuronal networks (3, 11, 12). A variety of studies are consistent with the idea that mechanisms similar to those of propagation of prion pathology could underlie disease progression. Prion protein (PrP)\(^2\) is a normal cellular protein that can be converted to a disease-causing conformation (PrP\(^{Sc}\)) through interaction with a pathogenic prion protein “seed.” The conversion mechanism is not fully understood, but involves templated conformational change, whereby a PrP\(^{Sc}\) seed contacts natively folded protein and induces it to assemble onto a growing aggregate. PrP\(^{Sc}\) aggregates can have multiple conformations, each linked to unique pathological patterns (13–15), and they have been demonstrated in experimental systems to propagate through neural networks (16). Thus, the prion hypothesis provides a useful model by which to test ideas about propagation of protein pathology in other neurodegenerative diseases.

Prion-like Propagation of Protein Pathology

Since the identification of prionopathies, many have theorized that conventional neurodegenerative diseases might occur by similar mechanisms. However, over the years there was no convincing evidence that this was true. With the identification of “slow virus” pathology by Gajdusek, a variety of studies attempted to create pathology by introducing brain homogenates from AD patients into experimental animals. There were variable reports of pathology (17–19), but because the studies lacked convincing controls, it was difficult to draw firm conclusions. Meanwhile, numerous studies began to draw parallels between PrP and other amyloid proteins in terms of fibril-forming behavior. For example, PrP exhibits “strain” phenotypes, which are defined by the stable propagation of unique pathogenic conformations in vivo. Unique properties of PrP\(^{Sc}\) structure are associated with different incubation times and pathologies in animal models (20, 21). This structure is replicated faithfully by templated conformational change in animal hosts. Many prion strains have now been propagated for decades in mouse models. Important studies of Aβ fibrils indicated that they have conformational diversity reminiscent of PrP\(^{Sc}\). Specifically, it was possible to create two distinct fibrillar Aβ conformers that propagate in vitro and that had different biological effects when applied to cells (22, 23). Similar properties were described for the Tau protein (24), and subsequently for α-synuclein (25, 26).

In vivo, there is now abundant evidence for seeding phenomena. Aβ has been most extensively studied. Brain homogenates from AD patients and mouse models of cerebral amyloidosis (PD-APP) produce Aβ pathology when injected into a host animal (27–29). Different sources of Aβ will produce distinct pathologies (30); small, soluble Aβ oligomers are especially potent inducers of pathology (31); and widespread cerebral β-amyloidosis was observed following inoculation of synthetic Aβ aggregates into mouse brain (32). All such studies are most consistent with the idea that pathology results from templated seeding reactions in vivo. These studies have convincingly dem-

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\(^2\) The abbreviations used are: PrP, prion protein; PrP\(^{Sc}\), cellular PrP; PrP\(^{Sc}\), disease-related PrP; AD, Alzheimer disease; Aβ, amyloid-β; HSPG, heparan sulfate proteoglycan.
onstrated that Aβ can template new pathological forms in vivo and that this is highly prion-like. However, because most Aβ pathology is extracellular, at a certain level this work could be explained simply by contact of Aβ seeds with extracellular Aβ peptide produced in the experimental animals. No cell-cell transfer of pathology was required to explain the findings, and thus these experiments could not fully account for the inexorable spread of many neurodegenerative diseases that are caused by intracellular protein accumulation.

Then, in 2008, two studies simultaneously reported on pathological studies of Parkinson disease patients who had received fetal dopaminergic cell transplants, in some cases as many as 15 years prior to death. These investigations observed that fetal-derived cells demonstrated α-synuclein accumulation reminiscent of Lewy bodies. This was remarkable because the cells were no more than 15 years old, which suggested that pathology might have derived from the host neurons (33, 34). However, it was still unclear whether this was due to a toxic “environment” within the host, or to actual protein transfer from one cell to another. A key test of this question was answered by experimentation in transgenic animals, in which wild-type mouse neural stem cells were implanted in mice transgenic for human α-synuclein. Human synuclein accumulation occurred in the transplanted neurons, which could only have happened due to cell-cell transfer (35–37). This work was accompanied by other studies, which demonstrated that aggregates of Tau protein were taken up into cultured cells where they could induce fibrillation of intracellular Tau (38, 39). Further, Tau aggregates newly formed in a cell were observed to transfer to co-cultured cells (38). This work was subsequently replicated by numerous groups for α-synuclein (36, 37, 40–43), SOD1 (44), huntingtin (45), and TDP-43 (46). It is now well established that protein aggregates are mobile and can transmit aggregates from cell to cell in vitro.

Work in animals has extended these studies in important new ways. Two studies have reported apparent trans-synaptic movement of Tau protein aggregates based on region-specific gene expression in a transgenic mouse line. In both studies, tetracycline-regulated gene expression was driven predominately in the entorhinal cortex, which projects axons to the hippocampus. In aged animals, aggregate pathology that was most likely to have derived from the entorhinal neurons was observed in the hippocampus (47, 48). Further, a recent study using a lentivirus-mediated rat model of hippocampal tauopathy demonstrated that wild-type Tau is transferred via axons to distant second order neurons (49). These studies strongly suggested that aggregated forms of Tau were moving across synapses, and thus could potentially explain the involvement of neural networks in neurodegenerative diseases. Finally, a recent study presents conclusive evidence that Tau stably propagates unique aggregate conformers, or “strains,” in cells and mice, and that human tauopathies are composed of disease-associated strains (101).

Other studies have shown that release of Tau monomer is increased by synaptic activity (50, 51). It remains unknown, however, whether trans-synaptic movement of aggregates is activity-dependent or whether it simply results from release of aggregated material at the axon terminal, where it is taken up by neighboring cells. Interestingly, extracellular Tau as well as transferred Tau species are largely dephosphorylated (49, 52–54), suggesting that hyperphosphorylated Tau aggregates are quite different from forms that propagate.

Antibodies to Target Pathology

In AD, significant protein deposition occurs in the extracellular space. A seminal study reported that vaccination against Aβ in transgenic mice that develop Aβ pathology could be profoundly beneficial (55). This opened the door to multiple studies of anti-Aβ vaccine strategies, both active and passive. Subsequent work targeted α-synuclein in a mouse model by a similar strategy. This produced demonstrable benefits, although at this time it was not appreciated that the vaccine might have worked by targeting extracellular α-synuclein (56). Since then, multiple active and passive vaccination studies have been carried out against Tau, with variable results (57–61). It is now fairly well accepted that antibodies against pathological proteins can ameliorate pathology in transgenic mouse models. Although the molecular mechanisms of antibody therapies are not yet determined, their efficacy strongly implicates extracellular protein in pathogenesis. Unfortunately, clinical studies of Aβ vaccines have failed to produce any benefit in the patients with AD (62, 63). Although Aβ may not be a good target for AD, more likely the treatment was initiated too late: in patients with moderate dementia who had already developed robust Tau pathology.

Because it is a practical impossibility to test all monoclonal antibodies in vivo, how will antibodies be prioritized? One strategy has been to identify the putative toxic form of a target protein, e.g. Aβ oligomers, and there have now been multiple antibodies and studies related to detection and targeting of these species (64). This approach has also been applied to Tau, in which an oligomer-specific polyclonal antibody was developed (58). Multiple active and passive vaccine studies have now targeted intracellular proteins (56, 57, 59, 60, 65–67), and most recently, a cell-based aggregate seeding assay was used to prioritize anti-Tau antibodies prior to testing in vivo (61). There are multiple potential mechanisms for any therapeutic antibody. These include alteration of Tau aggregate structure, e.g. promoting a disaggregation step or sequestering monomer; blocking uptake into neurons; promoting neuronal clearance or microglial uptake; and facilitating peripheral degradation. Importantly, complete genetic ablation of Tau is fairly well tolerated in mice, suggesting that anti-Tau antibodies in the adult CNS are unlikely to meet safety concerns due to disruption of normal Tau physiology (68–70). Finally, if parallels to prion disease hold true, there could be variable clinical responses in patients based on the conformation of the pathogenic species, and possibly evolution of protein aggregate structures away from a given therapy (71, 72). Although the most important criterion will be the efficacy of an antibody in vivo, a strong understanding of the biology of the most effective agents will enable better selection and optimization. Given the relative rapidity with which new humanized monoclonal antibodies can be created and the plethora of protein targets, it seems likely that more human clinical trials will result in the coming years.
Understanding Cell Uptake

If propagation of pathology from cell-to-cell underlies disease progression, then interruption of this process could be beneficial. At this point, it is not known how protein aggregates might exit a neuron. This could represent an adaptive response to an accumulation of intracellular aggregated protein. In this case, it might be counterproductive to block release. Conversely, intracellular aggregates might destabilize membranes to create transient rupture, or they might be released upon cell death or axon degeneration. Although much contention exists with regard to mechanisms of Tau secretion (52, 53, 73–77), a recent study on SOD1 aggregates suggests a dual mode of aggregate release. Under this paradigm, healthy cells release SOD1 into the medium in association with exosomes, whereas dying cells release free aggregates (78). However, there is clearly no consensus about release mechanisms involved in normal versus pathophysiology.

Initial studies of aggregate uptake implicated a role for “bulk,” or “fluid phase” endocytosis, but did not indicate a specific mechanism. One study has now defined the mechanism of cell uptake of Tau and synuclein aggregate seeds into neurons through macropinocytosis, a subtype of fluid-phase bulk endocytosis (79). Macropinocytosis involves dynamic actin restructuring, as well as the formation of large intracellular vesicles. This process is initiated by the binding of aggregated Tau and α-synuclein to heparan sulfate proteoglycans (HSPGs) on the cell surface. HSPGs constitute a family of core proteins that are decorated with glycosaminoglycan polysaccharides. These glycosaminoglycan chains are extensively sulfated, which specifies various interactions with extracellular ligands. Interestingly, although Tau monomer will bind these surface proteins via putative heparan sulfate binding domains, it will not initiate internalization, and only aggregated species trigger uptake through this mechanism (79). Finally, a new study suggests that HSPGs can mediate the internalization of exosomes (80). This might facilitate internalization of proteopathic seeds lacking heparan sulfate binding domains. As the HSPG pathway is better understood, it may be possible to design specific inhibitors to prevent aggregate entry and seeding in neurons, based on blocking Tau/HSPG interactions (Fig. 1).

A provocative parallel can be drawn between the cellular machinery mediating Tau aggregate internalization and seeding and that of virus infectivity. Indeed, virus internalization into eukaryotic cells often requires HSPG-mediated macropinocytosis (81–83). Thus, this pathway may serve as a common and generalizable mode of cellular entry for large particles, including pathogens.

It seems likely that other cells in the brain, e.g. microglia and astrocytes, could play a role in aggregate uptake and clearance. It is unknown whether cell type-specific mechanisms exist or whether common mechanisms apply. For example, although there is some evidence that an antibody can block Tau aggregate uptake into HEK293 cells (84), other studies indicate that an anti-synuclein antibody can promote α-synuclein uptake into microglial cells in culture (85). Thus, it will be important to dissect the biology of cellular internalization and the role of antibodies so as to promote targeting of aggregates toward clearance pathways (e.g. microglia) and away from trans-neuronal propagation.

Targeting of Cell Surface Proteins

HSPGs have been previously recognized for their association with amyloid plaques (86, 87), as well as for their ability to promote Tau protein fibrillization (88, 89). However, recognition of their role in protein aggregate uptake and intracellular seeding has presented interesting new possibilities for development of therapies. HSPGs are processed through a series of post-translational steps that add successive uronic acid and N-acetylglucosamine groups onto core proteins. A variety of sulfotransferases and epimerases further modify the maturing proteins. Specific interaction of HSPGs with extracellular proteins is created by unique sulfate patterns known as fine structure and enables proper cell signaling (90). It is unknown whether the interaction of Tau and synuclein seeds with HSPGs is specifically dictated by fine structure or whether nonspecific interactions such as electrostatics are sufficient. If the interactions are in fact specific, then it may be possible to design inhibitors of this pathway that selectively inhibit aggregate uptake and seeding into neurons without interfering with normal physiology.
Targeting Aggregate Binding to the Cell Surface

Tau aggregates bind and enter the cell via HSPIGS. This interaction can be blocked by heparin, by a variety of heparin mimetics, and by interfering with proper production and post-translational modification of HSPIGS (79). Importantly, HSPIGS have been previously linked to other disease-associated amyloids, including β-amyloid (91, 92), amyloid protein A (93), and prion protein (94–97). Thus, preventing proteopathic seeds from binding cell surface HSPIGS may serve as a viable target for slowing progression of multiple diseases. This approach has been tried for prion disease. Pentosan polysulfate is a large sulfated polysaccharide with weak heparin-like activity that is highly effective at inhibiting PrPSc formation both in vitro and in rodent models, and it is currently being tested in humans with Creutzfeldt-Jakob Disease (98–100). Although it is not clear whether this strategy will work, pentosan polysulfate was well tolerated and thus provides precedent for heparin-like molecules. Nonetheless, based on our growing understanding of pathogenesis, the basic biology has not yet been elucidated to the point where it is possible to begin target-based drug development. Although there are many clues to potential therapeutic mechanisms, the basic biology has not yet been elucidated to the point where it is possible to begin target-based drug development. Nonetheless, based on our growing understanding of pathogenic mechanisms, the path of drug discovery for neurodegenerative diseases has now become much more clear.

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