COMMENTARY

Expanding the prion disease repertoire

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In 1982, Stanley Prusiner proposed that the infectious agent of transmissible spongiform encephalopathies (TSEs), a group of relatively rare neurodegenerative disorders that includes Creutzfeldt–Jakob disease and scrapie, lacks replicating nucleic acids and instead is composed primarily of a misfolded conformation of the prion protein, which he termed PrP^{Sc} (1). Originally, the "prion" hypothesis was met with considerable skepticism because it was difficult to envision how an infectious agent could replicate without a nucleic acid genome. However, a variety of biochemical and genetic experiments subsequently demonstrated the existence of infectious prions (2), eventually fulfilling Koch's postulates (3). It is now generally accepted that infectious prions replicate through the autocatalytic misfolding of a normal host protein (PrP^{C}) into the PrP^{Sc} conformation. In PNAS, Prusiner et al. (4) report findings suggesting that another molecule, α-synuclein, might also act as a prion in a human disease.

Do Prions Cause Diseases Other than TSEs?

In recent years, many researchers have been investigating the intriguing possibility that a variety of other neurodegenerative diseases, including relatively common disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD), might also be caused by "prions" composed of misfolded proteins other than PrP^{Sc}. Although there is currently no epidemiologic evidence to suggest that either AD or PD has an infectious etiology, it has nonetheless been proposed, on the basis of neuropathological observations in human patients and experiments in cell and animal models, that specific disease-associated proteins such as Aβ, tau, and α-synuclein might also be prions that can spread progressively throughout the brain (5).

Following the model established by prior work on TSEs, the general experimental paradigm used in most of these studies has been to inoculate mice or primates with various inocula containing the candidate misfolded protein and then monitor the animals for the development of neurological symptoms and neuropathology. A realistic assessment of the results of these experiments would indicate that the new candidate prions seem to be much less efficient than PrP^{Sc} in causing neurodegeneration in normal hosts. Although all of the new candidate prions can form extensive deposits in brain tissue, this deposition typically does not cause neuronal death unless the host animal is engineered to express a pathogenic mutant protein (6, 7) or very large quantities of misfolded proteins are directly inoculated into a vulnerable brain region (8). Quantitatively, the new candidate prions seem to be over a millionfold less pathogenic than PrP^{Sc}, which can induce a transmissible disease when injected in subattomole quantities into nontransgenic hosts (9).

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The Case for Multiple System Atrophy Prions

Although templated protein misfolding could potentially play a mechanistic role in the progression of non-TSE neurodegenerative diseases, it is unclear what that role might be, given the relative inefficiency of the new candidate prions. Against this backdrop, Prusiner et al. (4) report striking results that shed light on how α-synuclein may act as a prion in human disease. Clinically, the deposition of misfolded α-synuclein in neurons or glial cells is the shared neuropathological characteristic that defines a diverse group of sporadic human neurodegenerative disorders that include PD, dementia with Lewy bodies, pure autonomic failure, and multiple system atrophy (MSA) (10).

Prusiner and his colleagues report that homogenates prepared from the brains of patients with MSA are able induce a disease characterized by motor symptoms in $Tg(M83^{+/-})$ mice, engineered to express human A53T α-synuclein (4, 11). This disease was accompanied by deposition of insoluble phosphorylated α-synuclein and was transmissible to $Tg(M83^{+/-})$ mice upon serial passage. In contrast, inoculation of brain homogenates from the brains of PD patients into $Tg(M83^{+/-})$ mice failed to induce either neurological symptoms or the deposition of insoluble phosphorylated α-synuclein (4). Prusiner et al. (4) also show that MSA, but not PD, brain homogenates could seed the intracellular aggregation of intracellular GFP-tagged A53T α-synuclein in cultured cells. The marked difference in seeding ability between MSA and PD brain homogenates in both assays is probably the most striking and significant aspect of the study. The results are particularly convincing because the investigators included 14 human cases of MSA and 7 cases of PD in their study. It is worth noting that the A53T mutation was originally linked to early onset PD with Lewy bodies in a Greek–Italian family (12), so one might have expected that it should have been easier for this mutant sequence to be seeded by PD brain homogenate than by MSA brain homogenate. The seeding specificity displayed in both the cell culture and animal assays provides compelling support for the hypothesis that α-synuclein prions might play a role in the pathogenesis of MSA. However, by the same reasoning, the results suggest that prions may not exist in PD brain. It is possible that a hypothetical "PD prion strain" might require an unknown experimental condition or host factor, but this specific condition must not be satisfied either in transgenic mice or HEK cells, and must also not be necessary for MSA prion conversion. An immediate practical application of the work of Prusnier et al. (4) is that the HEK cell assay can be used as a diagnostic test to distinguish MSA from PD.

A Few Unresolved Issues

Several issues remain to be resolved to rigorously evaluate the potential role of putative MSA prions in causing human disease. One key issue involves the apparent need to use the pathogenic A53T α-synuclein mutation in both cell and animal assays in order to

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detect seeding activity in MSA brain. Disturbingly, Prusiner et al. (4) report that inoculation of either MSA or PD prions into transgenic mice expressing WT human α-synuclein failed to induce neurological disease. This result raises the following question: If MSA prions are unable to template the aggregation of WT α-synuclein, are they relevant to the pathogenesis of sporadic disease in normal human brain where only WT, rather than mutant, α-synuclein is present? How might this concern be addressed experimentally? One possible explanation for the negative results with WT α-synuclein is that the transgenic mice used in these studies express α-synuclein exclusively in neurons, whereas in clinical cases of MSA α-synuclein typically forms inclusions within glial cells known as Papp–Lantos bodies. It is possible that, to be susceptible to prion-like conformational change, WT α-synuclein must interact with one or more specific cofactors that are present in glial cells but not neurons [notably, in a similar scenario, WT PrP^{C} requires specific cofactors to convert into PrP^{Sc}, but PrP molecules harboring pathogenic mutations can misfold without cofactors (13)]. To test this hypothesis, WT α-synuclein could be expressed in glial cells of transgenic mice by using either an endogenous synuclein promoter or a glial-specific promoter. If a specific component of the glial cell environment were necessary for the seeded conversion of WT α-synuclein, using a glial-specific promoter should allow that to happen.

A second issue involves the apparent lack of neuronal degeneration in symptomatic Tg($M83^{+/-}$) mice (4). The lack of neuronal death despite the presence of extensive insoluble phosphorylated α-synuclein deposits in neurons is notable because neurodegeneration is a central feature in the pathogenesis of clinical MSA. It is conceivable that the lack of neurodegeneration in Tg($M83^{+/-}$) mice might also be caused by the lack of glial α-synuclein expression, because glial cells might play an auxiliary role in mediating cell death. However, there may be a broader explanation for the lack of cell death in this and other models of human neurodegenerative diseases. It has been generally difficult to induce neuronal degeneration in a variety of rodent models of human disease simply by inducing protein misfolding in neurons. For example, the deposition of Aβ plaques in the brains of mice is not accompanied by cell death (6). Some logical explanations for this dissociation include the following: (*i*): Rodent neurons may be less susceptible than primate neurons to the effects of misfolded human proteins; (*ii*) it may take many years (more than the lifespan of a mouse) for neurodegeneration to occur; (*iii*) the conformations of aggregated proteins being propagated and deposited in these experiments may not be the pathophysiologically relevant (toxic) species; and (*iv*) neuronal degeneration may be a multifactorial process, and protein misfolding alone may not be sufficient to induce cell death. It is likely that different approaches will eventually be required to faithfully recapitulate neurodegeneration experimentally in model systems.

It will also be important to determine the potency of MSA prions. Prusiner et al. (4) inoculated Tg($M83^{+/-}$) mice with the equivalent ∼0.3 mg of MSA brain to induce disease but did not test lower doses. Typically, scrapie can be induced by inoculation of a millionfold lower dose into WT mice. It will be useful to measure the specific infectivity of MSA prions quantitatively by end-point titration for at least two reasons. First, confirmation of high specific infectivity would make it more likely that MSA prions are actually responsible for driving the progression of disease in human patients in a manner similar to PrP^{Sc} prions. Second, knowing the specific infectivity of MSA prions will help us assess their biohazard potential, so that appropriate precautions can be taken if necessary to prevent their iatrogenic spread.

In summary, roughly three decades after the seminal discovery of PrP^{Sc}, Prusiner and other investigators may be on the verge of expanding the prion disease repertoire to include non-TSE disorders such as MSA. However, critical work still remains to confirm the role of α-synuclein prions in the pathogenesis of neurodegeneration in sporadic MSA, and to identify which other neurodegenerative diseases might also be caused by novel prions. It is likely that continued research in this area will create exciting opportunities for developing targeted diagnostic and therapeutic tools based on the seeded propagation of specific proteins.

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