Misfolded, protease-resistant proteins in animal models and human neurodegenerative disease

Dennis W. Dickson

Departments of Pathology (Neuropathology) and Neuroscience, Mayo Clinic, Jacksonville, Florida


Neurodegeneration in the substantia nigra has been known to be a constant feature of Parkinson disease (PD) since the early part of the twentieth century, when Trétiakoff (1) correlated the clinical findings of patients with PD with postmortem pathology. A few years earlier, Lewy had described concentric hyaline inclusions within neurons, which are now referred to as Lewy bodies (LBs) (2). LBs are a sine qua non for the neurodegeneration that characterizes PD, but they are also found in nonparkinsonian disorders, most notably dementia with Lewy bodies (3). LBs are composed of filamentous aggregates of α-synuclein (4), and immunohistochemistry for synuclein is currently the most sensitive method for detecting LBs in brain tissue. Ubiquitin is another key component of LBs (5), and widespread use of immunohistochemical methods for detecting LBs, first with ubiquitin and later with synuclein antibodies, demonstrated that pathology in these disorders extended beyond LBs within neuronal perikarya to fibrillary lesions within neuritic processes, so-called Lewy neurites. Neuritic pathology is more widespread than LBs and affects regions of the brain, such as the hippocampus (6), that were not previously considered affected in PD. More recently, using antibodies to modified forms of α-synuclein (7) or sensitive antigen retrieval methods, it has been discovered that LBs are more prevalent and Lewy neurites more widespread than previously recognized. For example, LBs are present in a high proportion of Alzheimer brains (8), and the basal ganglia contain many Lewy neurites (7). The report by Neumann et al. in this issue of the JCI moves the field forward another step (9). The authors have employed a method for detecting abnormal forms of α-synuclein that may not necessarily be composed of fibrillar structures, which are the feature that permits ready detection of α-synuclein in LBs and Lewy neurites in tissue sections.

Histoblots detect protease-resistant protein

In this report (9) Neumann and coworkers applied a modification of a method, the histoblot (10), originally developed for detection of the pathologic form of prion protein (PrPres), which evidence suggests is the causative agent in transmissible spongiform encephalopathies such as Creutzfeldt-Jakob disease (11). The histoblot method takes advantage of the unusual solubility properties of PrPres (derived from the nonpathogenic cellular form of prion protein, PrP* that make it resistant to proteases. Sections of brain tissue are cut and applied to a nitrocellulose membrane support and subsequently treated with protease, which digests PrP* and leaves an imprint of the distribution of PrPres in the remnants of tissue adherent to the nitrocellulose. The nitrocellulose is then immunostained much like a Western blot, but the result is an immunohistochemical imprint of the pathologic form of the protein freed from the staining of normal cellular protein, which is abundant for PrP*. Instead of prion protein antibodies, Neumann and coworkers have used antibodies to α-synuclein, and instead of transmissible spongiform encephalopathy tissue, they have used Lewy body disease and other disorders, such as multiple system atrophy, with abnormal α-synuclein aggregates (Figure 1). As might be expected given the harsh treatment of the tissue, the method lacks fine resolution, but it does illustrate the general distribution of pathologic forms of α-synuclein without the confound of normal cellular α-synuclein, which is very abundant in synaptic termini in the neuropil of gray matter. The approach is not novel and has also been used to show abnormal forms of β-amyloid in human brains after ischemic stress (12). This is, however, the first use of a modification of histoblots to detect abnormal forms of α-synuclein.

In addition to studies of human tissue, Neumann and coworkers also mapped α-synuclein pathology in transgenic mice expressing human α-synuclein (9). The method proved to be very sensitive and specific for illustrating the distribution of the abnormal form of α-synuclein in the mouse brain, but the distribution does not map with the distribution of pathology in humans. A notable difference is that the midbrain tectum contained abundant protease-resistant α-synuclein but the substantia nigra contained hardly any. In humans, the reverse would be expected. The problems with this transgenic model are not unique. Initial attempts to generate α-synuclein transgenic mice that modeled PD met with limited success (13), but more recent models, including the one reported by Neumann and coworkers (9), have developed Lewy neurites and, occasionally, perikaryal inclusions that share some features with LBs from human brains (14, 15). Thus, α-synuclein transgenic mice are models for α-synuclein cytopathology,
forms of previous studies that found altered in, the study (9) confirms the results of
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tion of pathologic forms of α-synuclein. Beyond demonstration of the distribu-
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within the brain from a patient with Lewy body disease, immunostained for synuclein with no pretreatment and the histoblot method is that the histoblot may also detect a soluble, protease-resistant form of α-synuclein that would be washed away in the traditional immunos- 
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nonfilamentous form of α-synuclein is a natively unfolded protein with very little secondary structure (21). It is of more than passing interest that tau protein, the microtubule-associated protein that is the major structural component of neurofibrillary tangles in Alzheimer disease, shares a number of properties with α-synuclein (22). Tau is also a natively unfolded protein with little secondary structure that forms pathologic filaments with unusual solubility properties and protease resistance. Unlike the fibrillar forms of synuclein, however, tau appears to contain little β-sheet secondary structure (23).

The biochemical basis for the protease 
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unfolding of Neumann et al. (9), to interpret the effects of protease treatment as enhanced detection of pathologic forms of α-synuclein, because the normal cellular form is digested (Figure 2). The distinction between more widely used antigen retrieval methods and the histoblot method is that the histoblot may also detect a soluble, protease-resistant form of α-synuclein that that would be washed away in the traditional immunos- 
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sensitive (8, 18). While it is possible that protease treatment reveals hidden epitopes in α-synuclein, it is easier, especially in light of the observations of Neumann et al. (9), to interpret the effects of protease treatment as enhanced detection of pathologic forms of α-synuclein. Antigen retrieval and abnormal forms of α-synuclein

The biochemical basis for the protease resistance of α-synuclein and PrP⁰⁰⁰ is not entirely known, but most current evidence suggests that protein conformation may play a significant role. Creutzfeldt-Jakob disease is the archetype of a neurologic disorder caused by an abnormal conformation of a normal cellular protein. Comparative investigations of PrP⁰⁰⁰ and PrP have shown that conformation rather than posttranslational modification is the best explana-
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orthological filaments with unusual solubility properties and protease resistance. Unlike the fibrillar forms of synuclein, however, tau appears to contain little β-sheet secondary structure (23).

The characteristics of PrP⁰⁰⁰ and protease-resistant α-synuclein are also the characteristics of amyloid. Despite the
wide clinical diversity and pathogene-
sis of amyloidoses, they share common
properties, including increased fre-
cuency with age, defective proteolytic
processing, and association with acidic
macromolecules (24). The various amy-
lloid molecules are structural variants
of a normal precursor protein, and the
major structural feature common to
amyloid is β-sheet conformation, which
led Glenner to refer to these dis-
orders as the β-fibrilloses (25). There
are many paths to amyloid, but in most
cases the conversion of the normal pre-
cursor protein to the amyloid form of
the protein involves overcoming a ther-
modynamic energy barrier. The size of
the energy barrier and thus the likeli-
hood of conversion of the normal to
the pathologic form may be modified
by a number of factors, such as post-
translational modification (e.g., prote-
ylosis and phosphorylation) and asso-
ciation with chaperone proteins, lipids,
divalent cations, or acidic macromole-
cules. A fundamental difference
between prion diseases and the other β-
fibrilloses that remains to be explained
is that prion disease is an infectious or
transmissible fibrillosis, while none of
the other disorders has been shown to
be transmissible.

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