Commentary

Propagated protein misfolding: New opportunities for therapeutics, new public health risk

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Abstract

There is now good consensus that propagated protein misfolding is the underlying mechanism for the infectious prion diseases (Creutzfeldt-Jakob disease in humans, scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, and chronic wasting disease in deer and elk). Over the past decade it has become increasingly clear that other diseases, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis may progress via the same mechanism, involving a disease-specific polypeptide rather than the prion protein. Recent literature in these non-prion neurodegenerative diseases also points to the existence of multiple "strains" that express themselves differently in different contexts, resulting in different disease phenotypes. The probable cause of these neurodegenerative diseases is now referred to collectively as "propagated protein misfolding." Propagated protein misfolding raises many opportunities for new therapeutics and diagnostics. However, it also raises the theoretical risk of iatrogenic transmission, although experimental support for this notion is limited at present.

Introduction

The hypothesis that a misfolded protein could confer its misfold on a neighbouring normal protein and cause disease was widely regarded as heretical when first proposed in 1982 by Stanley Prusiner (1), but this idea is now well-accepted, and was sanctioned by a Nobel Prize in 1997. There is now good consensus that propagated protein misfolding of host prion protein (encoded in humans by the gene PRNP) is the underlying mechanism for the infectious prion diseases (Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease in deer and elk). What has become apparent more recently is the notion that misfolded proteins may be part of the underlying pathology in more common neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, and possibly some non-neurological conditions such as type II diabetes mellitus and peripheral amyloidosis (2).

The objective of this article is to provide some highlights of how non-prion protein misfolding is mediated through polypeptides in neurodegenerative diseases, to explore some of the opportunities this creates for both diagnosis and therapy, and to identify the theoretical risk this poses for iatrogenic transmission. See text box for common protein misfolding terminology.

Protein misfolding terminology

Epitope—for proteins, a region or sequence of amino acids which can be recognized by a specific antibody.

Exosomes—small (~100nM) membrane-bound vesicles which are secreted by living cells, containing protein and nucleic acids.

Prion—infectious aggregate of prion protein, responsible for transmission of CJD[1], scrapie, BSE[2] and chronic wasting disease.

Propagated protein misfolding—prion-like transmission of protein misfolding between cells in an organ such as the brain, mediated by protein aggregates and/or exosomes. Although prion-like, no evidence to date shows natural transmission of disease among or between species.

PPM[3] seeding—application of prion aggregation theory to propagated protein misfolding aggregates, requiring a specific event in which misfolded monomers aggregate into a productive template (slow kinetics) for recruitment of additional monomers (rapid kinetics).

PPM[3] strains—application of prion strain behaviour to non-prion propagated protein misfolding, correlating with differences in propagated structure of aggregates of implicated proteins.

Post-translational covalent modification—any change in a protein mediated by a chemical bond, such as oxidation and glycosylation.

[1]CJD = Creutzfeldt-Jakob disease   [2]BSE = bovine spongiform encephalopathy   [3]PPM = propagated protein misfolding

Misfolding prions and other polypeptides

We now know that propagated protein misfolding can occur either through infectious prions, composed of aggregated misfolded host prion protein, or through the propagated aggregation of other proteins implicated in neurodegenerative diseases (no natural infectious lifecycle). There is recent experimental proof, for example, that the following propagated misfolded proteins are now associated with the following neurodegenerative disorders: Amyloid-beta (Abeta) oligomers/fibrils in Alzheimer's disease (3); alpha-synuclein in Parkinson's disease and Lewy body dementia (4) and multiple systems atrophy (5); superoxide dismutase 1 (SOD1) (6) and transactive response DNA binding protein 43 (TDP43) (7) in amyotrophic lateral sclerosis, and tau in the tauopathies, as well as Alzheimer's disease (8). Both prion infection and non-prion neurodegenerative diseases progress through propagated protein misfolding.

Different propagated protein misfolding strains may drive different disease progression

Infectious prions (CJD, scrapie, BSE and chronic wasting disease) do not exist in one monolithic form, but rather can exhibit "strain behaviour." In sheep scrapie, for example, when the first infectious prions were transmitted to mouse models, approximately 20 strains were cloned—defined on the basis of incubation time, brain regions predominantly affected, and biochemical features such as glycosylation preferences. Very recently, it has been shown that non-infectious prion-like propagated protein misfolding agents causing neurodegenerative diseases also exhibit strain behaviour. In studies of Alzheimer's disease, strain properties of aggregated Abeta in human brain have been found to correlate with rate of progression of disease (9,10). In Parkinson's disease and in multiple systems atrophy, different strains of aggregated alpha synuclein have been identified in vitro and in vivo (11). In amyotrophic lateral sclerosis, the propagated misfolding of SOD1 has been shown to display at least two distinct forms in a mouse model of the disease (12). And tauopathy has been found to propagate in multiple strains (13) that correspond to the clinical features and rate of progression in human disease.

New therapeutics and diagnostics for protein misfolding diseases

A new drug development mindset is required for protein misfolding diseases. For over a century, pharmacological science has been highly successful in applying small molecules to fixed structures: this has been an effective strategy for selective targeting of channels, pores and enzyme surface pockets and pits that can induce change in protein activity. However, the small molecule approach does not generally apply in protein

misfolding where the targets are often large and unstable. Moreover, propagated protein misfolding diseases involve protein−protein interactions which have proven to be very difficult to target with drugs.
The identification of strains provides opportunity for "precision medicine" treatment of neurodegenerative diseases. But there is a downside: data have accumulated indicating that treatment of one strain of infectious prions can "select" for another strain that can be more pathogenic. With this precedent from infectious prions, we should be aware that blocking propagated protein misfolding in other neurodegenerative diseases could also select for emergent strains that will require their own specific treatment.

Our own work has envisioned that effective treatment and diagnosis of protein misfolding diseases will require a new paradigm based on the rational identification of selective epitopes (or antibody targets) in the misfolded proteins that are key to the disease process. Selective antibody targeting of misfolded proteins is effective by several mechanisms, including the neutralization of aggregate cytotoxicity and inhibition of prion-like propagated protein misfolding (14).

In some conditions, such as infectious prion disease and Alzheimer's disease, the specific targeting of misfolded propagating proteins is like finding "a needle in a haystack" where the misfolded species may be present in a thousand-fold to a million-fold lower concentration than the natively folded species—a situation we have dubbed "target distraction." But the advantage of specific immunotherapy for misfolded proteins is that it would spare normally folded protein isoforms from autoimmune recognition.

Misfolding-specific epitopes can be produced by gain or loss of structure

Misfolding epitopes for immunotherapy or disease biomarkers can appear by two mechanisms in protein misfolding diseases: a gain or loss of structure not present in normal protein isoforms. Gain of structure can occur through generation of neoepitopes associated with post-translational covalent modification (mediated by the formation of a chemical bond). Non-covalent forces can also engender neoepitopes. For example, we have identified new gain-of-structure epitopes generated by non-covalent forces underlying specific aggregate morphologies, such as Abeta oligomers in Alzheimer's disease (15).

Loss of structure can occur when a structured domain of a protein "loosens" revealing a linear sequence that can be recognized by an antibody generated against the free peptide sequence. We have developed rational methods for predicting disease-specific epitopes caused by loss of structure, for which the outputs are sequences of linear peptides that go from structured to unstructured in the misfolding protein, against which antibodies can be generated and screened against the target peptide in the context of the protein misfolded in disease. For example, we identified misfolding-specific epitopes in SOD1 by both hypothesis generation and computational approaches, to block the toxicity and prion-like propagation of SOD1 misfolding in amyotrophic lateral sclerosis (16,17).

Theoretical risk of iatrogenic transmission

There is no doubt that true infectious prions can be detected in the blood and are competent to transmit disease (18); indeed, for variant CJD there have been five well-documented transmissions of disease through blood or blood products (19). However, the presence of protein misfolding seeds in non-prion propagated protein misfolding neurodegenerative diseases has been less clear. Only one journal report of blood Abeta oligomers in Alzheimer's disease has been published (20).

A few scientific reports (including scientific presentations at meetings and patent applications) suggest that transmission of Alzheimer's disease pathology can occur through bloodborne transmission in experimental animals. Alpha synuclein oligomers have been detected in the plasma of patients with Parkinson's disease, but are also observed in similar levels in normal controls, suggesting that the detected analyte is not pathogenic (21). No reports exist of SOD1 or TDP43 oligomers in the peripheral blood or serum, perhaps due to inadequate sensitivity and/or the transmission of bloodborne seeding activity through alternate pathways, such as exosomes (17). Interestingly, a single report shows that individuals injected with cadaveric growth hormone have a higher incidence of amyotrophic lateral sclerosis, but not Alzheimer's disease or Parkinson's disease (22). Thus, although a theoretical risk may pertain to iatrogenic transmission of neurodegenerative diseases, little experimental and epidemiological work supports this.

Conclusions

A new era in diagnosis and treatment of propagated protein misfolding diseases is upon us, offering many opportunities to treat neurodegenerative diseases that were previously untreatable or poorly treatable. Specific immunotherapies to block propagation and toxicity of misfolded proteins hold potential for treatment. The spectre of iatrogenic transmission of amyotrophic lateral sclerosis, Alzheimer's disease or Parkinson's disease and other neurodegenerative syndromes is a theoretical risk, but there is little to no support for seeding species of non-prion propagated protein misfolding diseases being detectable in peripheral blood, or experimental paradigms demonstrating this unsettling prospect at the present time.

Conflict of interest

Dr. Neil Cashman is the Canada Research Chair in Neurodegeneration and Protein Misfolding Diseases and is the Founder and Chief Scientific Officer of ProMIS Neurosciences Inc.

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