Human Prion Diseases (Part 1)
Structure, Functions, and Genetics of Prions

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Abstract: Prion diseases, or transmissible spongiform encephalopathies (TSE), are a group of neurodegenerative disorders, which affect humans and animals. Human prion diseases appear as a result of inherited, sporadic, and acquired manifestations. The main pathological feature of these disorders implicates the structural alteration of prion protein (PrP). It is believed that these disorders are associated with the accumulation of an abnormal isoform (PrPSc) of the normal cellular prion protein (PrPC). Several aspects of these disorders, including the classification, structure, functions, and genetics, were reviewed.

Keywords: Creutzfeldt-Jakob disease, transmissible spongiform encephalopathies
Introduction

In the past 10 years, prion diseases have attracted public and government attention, due to their role as the causative agents of ‘mad cow disease’. Prion diseases, or transmissible spongiform encephalopathies (TSE), are a group of human and animal neurodegenerative disorders. These disorders share common etiology and pathogenesis, which involve the structural alteration of prion protein (PrP). These chronic and fatal diseases consist of inherited, infectious, and sporadic subtypes. In human, prion diseases manifest in four subtypes, including Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträusler-Scheinker syndrome (GSS syndrome), kuru, and fatal familial insomnia (FFI). These disorders demonstrate clinical features such as dementia (except FFI), akinetic mutism, ataxia, motor dysfunction, sight loss, and other variable symptoms of central nervous system disturbances. Electroencephalograms may show generalized, synchronous, and periodic waves. Deaths may occur rapidly within months or even weeks. Unfortunately no effective therapy for this fatal disease has been found.

It has been suggested that prion diseases are caused by an agent termed “prion” (proteinaceous infectious particles). This agent is an unconventional infectious pathogen that cannot be classified as virus or viroid. Regardless the route of the prion agent in order to manifest in human, either through inheritance, sporadic, or iatrogenic/acquired transmission, these disorders are characterized with the accumulation of an abnormal isoform (PrPSc) of the normal cellular prion protein (PrPC). It is thought that PrPC is converted to PrPSc through the unfolding of alpha-helices and refolding into beta-sheets. To present, however, the nature and the pathophysiology of this nucleic acid deficient agent remain to be the conundrum of many studies.

This essay describes several aspects of prion diseases with the focus on the overview classification, the structure, the characteristic, the function and the genetics of prion diseases. In this essay, the term PrPSc is used interchangeably with the term PrP (protease ‘resistant’ prion protein), referring to the term used by the original articles, although this might be inappropriate for depicting the protease resistance characteristic of PrPSc. Another review on the pathogenesis of prion diseases will follow in a separate manuscript.

Classification of Prion Diseases

Prion diseases affect both humans and animals. In animals, it has been reported that these diseases only appear among mammalian species. The animals prion diseases include six varieties, namely scrapie (in sheep and goats), transmissible mink encephalopathy (mink), bovine spongiform encephalopathy (BSE) or ‘mad cow disease’ (cattle), chronic wasting disease (mule deer, elk), feline spongiform encephalopathy (cats), and exotic ungulate encephalopathy (ante-

lopes). The humans prion diseases fall into four major categories, i.e. Creutzfeldt-Jakob disease (CJD), which is brought about by iatrogenic, sporadic, familial, and variant causes; kuru; Gerstmann-Sträusler-Scheinker syndrome (GSS syndrome); and fatal familial insomnia (FFI). Human prion diseases appear as a result of inherited, sporadic, and acquired manifestations. Sporadic Creutzfeldt-Jakob disease appears as the most frequent form of human prion disease. Autosomal dominant inherited forms occur in 15% of all cases. Less frequent forms of prion diseases manifest as a consequence of iatrogenic routes, such as treatment with duramater or corneal transplantation, growth hormone of the pituitary human cadaver, and exposure to non-sterile neurosurgical equipments. The vast majority of prion diseases are the sporadic form with unknown etiology. It is thought that somatic mutation of PRNP (prion protein gene) or spontaneous transformation of PrP (normal prion protein) to PrPSc (abnormal scrapie isoform) underlies the manifestation of the sporadic forms.

Prions Structure and Characteristic

In the early 1980s, a new prion protein agent has been reported to be associated with scrapie infections. These small proteinaceous infectious particles, which was later termed “prion”, differ from viruses and viroids since they do not have any nucleic acids, neither DNA nor RNA. These prions are very small in dimension, the smallest ones of which are 100 times smaller than the smallest virus.

Prions are mainly composed of a protease-resistant sialoglycoprotein which has molecular size of 27-30 kDa (PrP 27-30). PrP comprises two forms: PrPSc and PrP. PrPSc is protease sensitive protein with a molecular weight of 33-35 kDa, while PrP is composed of a protease-resistant protein (PrP 27-30). PrPSc is an insoluble derivative of a N-terminal cleavage of PrP. PrPSc is formed from PrP by post-translational modification. Both PrPSc and PrPSc have been observed to have identical amino acid sequence, which consists of N-terminal sequence formed by the loss of a 22-amino-acid signal peptide (see Figure 1). This, in spite of the sequence of PrP, could be various in different species or even in the same species. There are two variants in humans, the M type that contains methionine, and the V type that is made up of valine in position 129. It is predicted that the tertiary structure of PrPSc is a four-helix bundle with two parallel pairs of alpha-helical overlapping segments. An important difference between the normal and the abnormal isoform can be seen in their tertiary structure. PrPSc contains mostly alpha-helical structure (42%) with a small percentage of beta-pleated sheets (3%). In contrast, PrPSc and PrP 27-30 are formed with higher percentages of beta-sheet-like form, namely 43% and 54%, respectively (see Figure 2). The alpha-helix constitutes 30% of PrPSc structure.
in various tissue but it is prominent in the central nervous system and in lymphoid tissue. It can also be observed in the liver, pancreas, kidney, mammary gland, and skeletal muscle.\textsuperscript{1,3,5,8,11,16,18}

This normal isoform is produced in the endoplasmic reticulum, and distributed through the Golgi apparatus to the cell membrane, where it is engaged to a glycophosphatidylinositol (GPI) and a cell surface proteoglycan. PrP\textsuperscript{C} is formed slowly, degraded rapidly, released after treatment with phosphatidylinositol-specific phospholipase, and entirely disintegrated by proteinase K digestion. Its half-life is very short, approximately 3-6 hours.\textsuperscript{5,11-13,16} PrP\textsuperscript{C} is transported along axons, and it is piled up at the neuromuscular junction.\textsuperscript{9}\textsuperscript{,}19 On the other hand, PrP\textsuperscript{Sc} is produced slowly (but probably more rapid than the formation of PrP\textsuperscript{C}) by a post-translational process. PrP\textsuperscript{Sc} is degraded slowly and is not released following phospholipase exposure. It is also partially cleaved and liberates PrP 27-30 after protease treatment by removing 66 NH\textsubscript{2}-terminal amino acids. PrP\textsuperscript{Sc} is believed to be more stable than PrP\textsuperscript{C}. It is stored mainly in the secondary lysosomes. PrP\textsuperscript{Sc} can be found in endosomal vesicles and on the cell surface. Neuronal degeneration might be due to the deposition of these proteolytic-enzyme-resistant PrP\textsuperscript{Sc} in synapses.\textsuperscript{6,12,16,21} The rate of PrP\textsuperscript{Sc} formation seems to correlate with the degree of PrP\textsuperscript{C} expression, whereas the rate of PrP\textsuperscript{Sc} deposition does not correspond to PrP\textsuperscript{C} level.\textsuperscript{6}

**Figure 1.** Tertiary structure of cellular prion protein which is inserted into a membrane lipid bilayer (E). (A) an alpha helix, (B) a beta sheet, (C) N-terminal, (D) glycosyl phosphatidylinositol (GPI) anchor. Reproduced from Aguzzi & Heikenwalder (2006)\textsuperscript{3} with permission from the publisher, with a slight modification.

Prions are characterized with their high resistance against heat, many chemical substances, such as formalin, alcohols, detergents, disinfectants, stomach enzymes (therefore they can survive in gastrointestinal tract and penetrate the lymphoreticular systems); DNase or RNase, psoralens, hydroxylamine, and photochemical reactions (ionization and ultraviolet or gamma radiation). However, they can be paralyzed with a strong base liquid or proteinase.\textsuperscript{1,3,5,7,12,16}

**The Function of Prions**

To date the exact function of PrP\textsuperscript{C} remains unknown.\textsuperscript{1-3,5,7,11,13} It has been thought that this protein is involved in activating lymphocyte, acts as a growth factor for neurons, assists synaptic functions, and induces or protects cells against apoptosis. It may also play a role in metal ions trafficking, cell adhesion, cellular protection against oxidative stress, copper and zinc metabolism, and signal transduction.\textsuperscript{2,3,11,12} It has been shown that in mice lacking PrP gene, the neuromuscular junction functioning is normal, but the neuronal and synaptic functions in hippocampus may be disrupted. However, other studies on hippocampus and cerebellum failed to demonstrate these abnormalities.\textsuperscript{19}

PrP is likely to play a pivotal role in the functioning of inhibitory synapses, especially in GABA transmission. This is based on the finding that in mice with inactivated gene encoding PrP, demonstrated an attenuated GABA\textsubscript{A} receptor-mediated fast inhibition in hippocampus. This suggests a
possible postsynaptic function of PrP. Another finding in similar mice revealed extensive degeneration of Purkinje cells in cerebellum, which are believed to produce large quantity of PrP and use GABA in inhibitory signaling. The Purkinje cells loss is probably a consequence of persistent over excitation due to the absence of PrP. The alterations of circadian rhythms in this type of mice substantiate this notion. Circadian rhythms are considered to be regulated by suprachiasmatic nucleus of the hypothalamus, and the production of GABA receptor subunits is high in this nucleus.

However, the importance of PrP is questioned since it has been shown that mice lacking PrP gene underwent a normal development, although they died prematurely without signs of specific disease.

**Genetics of Prion Diseases**

PRNP, the human PrP gene, which is engaged in the short arm of chromosome 20, provides a single copy encoding the 253-amino acids PrP. The mutations of PRNP in the familial subtypes of CJD, GSS, and FFI (all of which are characterized with autosomal dominant inheritance) involve two ways: a) point mutations, and b) insertions which integrate the enhanced number of octapeptide repeats. Studies on PRNP reported mutations at codons 102, 105, 117, 145, 178, 180, 183, 198, 200, 208, 210, 217, 232, and insertions of 2, 4, 5, 7, 8, or 9 octarepeats. The mutations are also associated with insertions at 144-base-pair (six extra octapeptide repeat) and 96-bp (additional four octapeptide repeat) in the prion protein gene. It is believed that one point mutation at codons 105, 117, 198, and 217 are always related to valine 129, whereas codon 102 correlates with the methionine 129. All of the mutations are considered to give rise to structural alterations of PrP protein from alpha-helical configuration to beta-pleated sheet.

Familial CJD is signified with the insertion of octarepeat sequences or point mutations at codon 178, 200, 208, or 210. The point mutation at codon 200 brings about a replacement of a glutamate (E) to lysine (K) (E200K). The mutation at the second nucleotide of codon 208 substitutes guanine to adenine, which results in a non conservative replacement of arginine to histidine at residue 208 of the protein (R208H).

Since there is no particular PRNP mutation observed in sporadic CJD, it is unknown how this subtype manifests. Nevertheless, the majority of sporadic CJD patients share the feature of PrP polymorphism of the amino acids methionine (Met) and valine (Val) at codon 129. The increased risk for CJD manifestation and the reduced duration of the disease compared to the heterozygous form are thought to be associated with the homogygosity of Met/Met. This results in the conversion of PrP to PrPSc. This occurs in all cases of the new variant of CJD. The homogygosity of valine at PrP codon 129 has also been observed in iatrogenic CJD cases. Both iatrogenic and sporadic subtypes of CJD are likely to appear in subjects with certain genetic predispositions, related to ordinary polymorphism at codon 129. The proportion of homozygosity of methionine allele is approximately 38% among Caucasians, while that of valine allele and heterozygosity are about 11% and 51% respectively. Homozygous subjects, either with Met or Val at the polymorphic codon 129, were associated with the largest proportion of sporadic and pituitary-hormone related iatrogenic CJD. This suggests that the homogygosity might be a predisposing factor. Nevertheless, in the Japanese population, the proportion of the Val allele is much lower. CJD cases with heterozygosity at codon 129 (M/V) are more common (18%) in the population, which has the proportion of 0% for V/V, 92% for M/M, and 8% for M/V.

Inherited disease with autosomal dominance and full penetrance were found in nearly all GSS syndrome patients. The most ordinary genetic subtypes of GSS are identified with the replacement of proline to leucine at codon 102 of the PrP gene (ataxic GSS). Other mutations occur at codons 117 (telencephalic GSS), 145, 198, and 217.

On the other hand, a rapid progressive autosomal dominant inheritance with incomplete penetrance was observed in FFI cases. Although FFI differs from familial CJD in neuropathological and clinical findings, FFI resembles familial CJD in the point mutation at PrP gene codon 178 (aspartic acid to asparagine mutation). FFI is characterized with either polymorphic homogygosity or M/V heterozygosity at codon 129. This disorder involves a PrP allele with methionine at codon 129 and asparagine at codon 178 (D178N-129M); while familial CJD shows a combination of a PrP allele with valine at codon 129 and asparagine at codon 178. FFI with homogygosity at codon 129 is associated with short duration disease, whereas that with heterozygosity corresponds with long duration disease. It should be noted however, that the clinical and pathologic characteristic of FFI may vary widely, and this disorder cannot be discriminated easily from sporadic CJD on clinical basis only. The diversity of clinicopathologic features of FFI designates the less tight association between genotype and phenotype. It seems that this various manifestation correlates with other factors, rather than the non-pathogenic polymorphism at codon 129. For instance, it was reported that pathogenic lysine mutation at codon 200 associates with pronounced insomnia.

It seems that the classical classification of human prion disease, covering the four subtypes (CJD, GSS, FFI, and kuru), is not sufficient in delineating the more complex variability of neuropathological changes. For instance, in the cases of mutation at codon 183 involving an adenine to guanine transition at nucleotide 547 and bringing about non-conservative substitution of threonine by alanine in the PrP protein, the clinicopathological features could not be categorized into the available subtypes.

**Conclusion**

Several facets related to the characteristics of the caus-
ative agent of prion diseases have been described, leaving a number of unsolved puzzles. Many problems associated with the fundamental aspects, such as the function of prion proteins and the relationship between genetic mutation and pathological manifestations of prion diseases remain unanswered.

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References