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PRION PROTEIN IN THE INFLUENCE OF ALZHEIMER'S DISEASE

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ABSTRACT

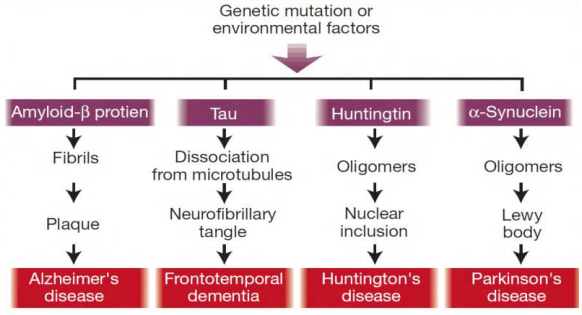
Human diseases characterized by insoluble extracellular deposits of proteins have been recognized for almost two centuries. Particularly challenging examples of such disorders occur in the post-mitotic environment of the neuron and include Alzheimer's disease. Aggregation of misfolded proteins that escape the cellular quality-control mechanisms is a common feature of a wide range of highly debilitating and increasingly prevalent diseases. A unified view of the molecular and cellular pathogenesis of these conditions has led to the search for chemical chaperones that can slow, arrest or revert disease progression. The primary biological function of the endogenous cellular prion protein has remained unclear. Drug resistance is a refractory barrier in the battle against many fatal diseases caused by rapidly evolving agents, including HIV, apicomplexans and specific cancers. Emerging evidence suggests that drug resistance and recurrent viral infections might extend to lethal prion disorders and related neurodegenerative amyloidoses.

KEYWORDS: Alzheimer's Disease (AD); Prion Protein; Neurodegeneration; Prionopathy; amyloidoses

INTRODUCTION

In the last decade, protein aggregation has moved beyond being a mostly ignored area of protein chemistry to become a key topic in medical sciences [1], mainly because the presence of insoluble deposits in human tissues correlates with the development of many debilitating human disorders including the amyloidoses and several neurodegenerative diseases [2]. Toxic insults resulting from biochemical or genetic accidents might trigger neurodegenerative diseases by co-opting apoptotic signaling pathways, for example through free-radical generation or caspase activation. An emerging theme in adult neurodegenerative disorders is the toxicity of abnormal protein structures or aggregates, which might be important in the pathogenesis of Alzheimer's disease (AD), Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (Fig. 1) [3].

Figure 1 Abnormal protein structures and the pathogenesis of neurodegenerativedisease. Normal proteins might become pathogenic when subjected to genetic mutations or environmental factors that promote the formation of abnormal structures in specific neuronal subpopulations.



Alzheimer's disease (AD) is one such type of very common form of dementia (neurodegenerative disease) which currently affects more than 37 million people worldwide [4]. AD is the sixth leading cause of death in the United States and remains one of the only causes of death that increased by as much as 66% over the last decade [5]. The prevalence of AD will increase further with an aging population, bringing wide social and economic demands for the care and treatment of AD patients. Plaques and tangles in the brain are two main features of AD. The third is the loss of connections between nerve cells neurons in the brain [4].

The Prion Protein is involved in neurodegeneration via its conversion from the normal cellular form, PrP^{c}, to the infectious form, PrP^{sc}, which is the causative agent of the Transmissible Spongiform Encephalopathies (TSEs). PrP^{c} has also enthralled scientists because its function in the body is ambiguous [6]. PrP^{c} appears to be important in preventing AD, since it inhibits β-secretase from creating the beta-amyloid plaques that cause the disease [7].

The cellular prion protein (PrP^{C}) is a cell membrane-bound glycoprotein with a molecular weight of 33–35 kDa. Present in various organs, it is especially abundant in the central nervous system (CNS) [8, 9]. One of the first studies on the inmunoreactivity of PrP^{C} in the human brain is that of Esiri et al [10]. Little is known about the physiology of PrP^{C}. PrP^{C} involvement in phenomena such as adhesion, neuroprotection and cellular signaling has been noted [11]. The two isoforms, PrP^{c} and PrP^{sc}, only differ in conformation and have the same primary amino acid sequence [12]. This along with the absence of nucleic acid in the prion protein, suggests that the conversion occurs post-translationally. While PrP^{c} is composed of 42% alpha-helix and only 3% beta-sheet, PrP^{sc} is composed of 30% α-helix and 43% β-sheet (Fig.2) [12]. Various models have suggested that PrP^{c} converts to its pathogenic isoform when the region corresponding to the residues 108-144 fold into beta-sheets [12]. Binding of Cu^{2+} also promotes the conformational shift from a predominantly α-helical to a β-sheet structure [13].

The N-terminal region of human PrP contains four sequential copies of the highly conserved octarepeat sequence PHGGGWGQ spanning residues 60−91. There is extensive cooperativity between different binding sites in the protein. The two highest-affinity binding events occur at the fifth site and at the octameric repeat region. However, the first binding is that to the octameric repeat region. Subsequent binding events after the two initial binding events have lower affinities within the octameric repeat region [14].

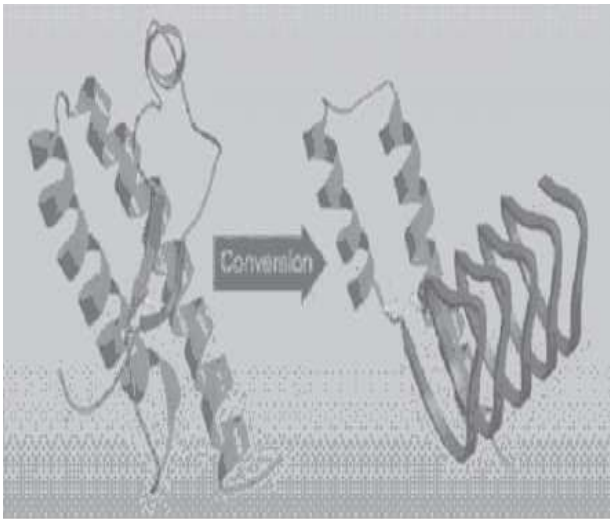
This region selectively binds Cu^{2+} in vivo. It has been shown that copper stimulates PrP endocytosis. The identified Gly−Cu linkage is unstable below pH ≈6.5 and thus suggests a pH-dependent molecular mechanism by which PrP detects Cu^{2+} in the extracellular matrix or releases PrP-bound Cu^{2+} within the endosome. The structure also reveals an unusual complementary interaction between copper-structured HGGGW units that may facilitate molecular recognition between prion proteins, thereby suggesting a mechanism for transmembrane signaling and perhaps conversion to the pathogenic form [15].

Lorca et al. study the effect of full-length PrP^{c} in Cu^{2+} inhibition of P2X4 receptor when both are co-expressed. PrP^{c} expression does not significantly change the ATP concentration-response curve in oocytes expressing P2X4 receptors. The presence of PrP^{c} reduces the inhibition of Cu^{2+} of the ATP-elicited currents in these oocytes. These observations suggest a role for PrP^{c} in modulating synaptic activity through binding of extracellular Cu^{2+} [16].

Because of many structural differences, the two isoforms have different biochemical characteristics. PrP^{c} is very soluble in detergents and easily digested by proteases while the PrP^{sc} is insoluble in detergents and resistant to protease digestion [12]. Current research hypothesizes that

PrP^{sc} inhibits the 26S proteasome, thus resisting degradation [17]. Therefore, a method used by researchers to differentiate between PrP^{c} and PrP^{sc} is to treat the proteins with a proteinase-K digest [18]. PrP^{sc} is only partially digested, generating a 27–30 kDa fragment. PrP^{sc} is only present in the prionopathies, and its brain concentration is 10 to 20 times higher than that of PrP^{c} in healthy brains [19].

Figure 2: Structural Differences Between PrP^{C} and PrP^{Sc}.
Left: PrP^{C} with only 3% β-sheet but 42% α-helix.
Right: PrP^{Sc} with 43% β-sheet and 30% α-helix.



Prion diseases exist in infectious, sporadic, and genetic forms. The infectious prion diseases are speculated to result from a spontaneous conversion of endogenous PrP^{c} to PrP^{sc} or following the introduction of PrP^{sc} in the body [20]. Based on previous studies carried out in various animals, and knowing the extrapolation is legitimate, J.L. Velayos et al. [19], proposed a retrograde spread of modified prion proteins from the zone of entry into the nervous system. An increasing number of researchers have also concluded that the spread is retrograde [21, 22, 23].

Prion diseases can also be inherited [24]. Prion diseases induce death when the PrP^{sc} accumulates in the brain, resulting in neuronal death and the creation of microscopic holes, known as vacuoles, in the brain [6]. A common polymorphism occurs at codon 129 of the PRNP gene, where either metionine or valine can be encoded. This codon is thought to have a role in determining the susceptibility of a person to prion diseases [6]. It has been hypothesized that while homozygosities for metionine or valine may increase the susceptibility to prion diseases, a hetrozygote may be protected from them. This is significant since the hypothesis suggests that while 50% of the Caucasian population might be at risk, 50% are not [25]. This implies that the interaction between two homozygous prion proteins is stronger than the interaction between two heterozygous prion proteins.

Furthermore, the homozygotes and hetrozygotes at codon 129 experience different disease phenotypes. Those with 129MM normally display a shorter, more rapid course of disease with cognitive distortion, while 129MV and 129VV cases present a longer course of disease, along the ataxia [18]. The polymorphism at codon 129 has been linked to other serious neurological disorders. A methionine homozygote has been reported to show an increased risk to late-onset AD [18].

Recently it was shown that a 21-residue fragment of the prion protein (106-126) could be toxic to cultured neurons. This peptide forms ion-permeable channels in planar lipid bilayer membranes. These channels are freely permeable to common physiological ions, and their formation is significantly enhanced by "aging" and/or low pH. It is suggested that channel formation is the cytotoxic mechanism of action of amyloidogenic peptides found in prion-related encephalopathies and other amyloidoses. The channels reported here are large and nonselective enough to mediate cell death through discharge of cellular membrane potential, changes in ionic homeostasis, and specifically, influx of calcium, perhaps triggering apoptosis [26].

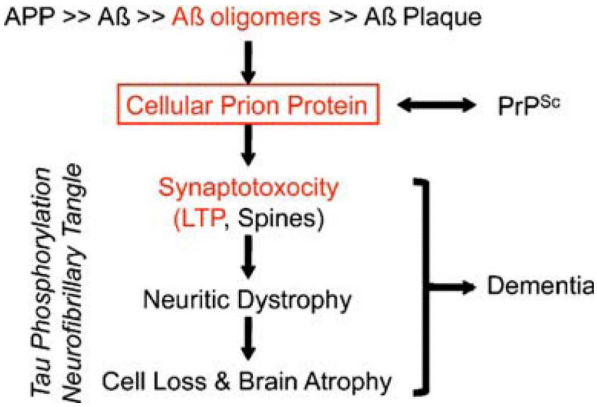
These findings suggest that the prion protein may play a role in these disorders, and that its function

may soon be elucidated. The findings that both PrP^{sc} and beta-amyloid peptide potentially bind PrP^{c} as oligomers, and both are principle suspects in progressive neurodegenerative disorders, raise suspicion of a common downstream mechanism. Decreased cerebrospinal fluid PrP levels correlate with increased disease severity in AD as well as other neurodegenerative disorders [27, 28]. A study conducted by Byron Caughey et al. on PrP-res isolated from the brains of Hamsters infected with hyper, drowsy, and 263K TSE strains indicates the support of hypothesis that strain-specific PrP-res conformers can self-propagate by converting the normal prion protein to the abnormal conformers that induce phenotypically distinct TSE disease [29].

Mouse genetics models expressing familial AD mutant APP with or without familial AD mutant PSen1 are useful tools for the laboratory study of AD. However in these models cognitive decline occurs without the widespread neurodegeneration observed in human AD [30]. Although the mice exhibit histological changes such as a reduction in serotonin immunoreactivity [31], the central human AD pathologies of reduced brain volume, hyperphosphorylated tau tangles, and widespread neuronal death do not result from expression of these transgenes.

The memory impairment of AD is due at least in part to synaptic impairment that is upstream in a pathway leading to neuronal death (Fig. 3) only under permissive or cooperative circumstances not present in transgenic murine physiological or temporal conditions. In support of this model, neutralization of the neuroprotective effects of secreted amyloid precursor protein in APPswe mice results in tau phosphorylation and loss of hippocampal neurons [32], presumably by unmasking the neurotoxic effects of Aβ42.

Fig. 3: Model of Aβ42 action through PrP^{C} receptor. APP processing yields Aβ monomers, which undergo oligomerization eventually forming amyloid plaques. Aβ oligomers bind to PrP^{C}, inducing LTP inhibition. The oligomeric infectious form of PrP and PrP^{Sc} independently induces neurodegeneration in a PrP^{C}-dependent fashion.



These mouse models provide a means to specifically examine the memory deficit mechanisms of AD in the absence of attendant neurodegeneration and will be valuable tools for exploring the function of Aβ42 oligomer/PrP^{C} interaction as it relates to the onset of memory impairment in AD. Knocking down levels of the endogenous prion protein PrP^{c} is a potential strategy for preventing neuronal damage in prion diseases. However, to date this principle has primarily been demonstrated in vitro or by creating transgenic mice in which PrP^{c} is deleted-an approach that cannot easily be translated to the clinic [33].

PrP is required for the plasticity-impairing effects of *ex vivo* material from human AD brain and that standardized Aβ-derived diffusible ligand (ADDL) preparations disrupt hippocampal synaptic plasticity in a PrP-dependent manner. Two representative and extensively characterized monoclonal antibodies directed to the principle PrP/Aβ-binding sites, ICSM-35 and ICSM-18, were shown to block the mediated disruption of synaptic plasticity validating these antibodies as candidate therapeutics for AD either individualy or in combination. As both ADDL preparations and Aβ extracted from human brain in aqueous buffer are highly heterogeneous, additional studies are required to biophysically characterize the key toxic species that bind to PrP [34].

In order to control the spread of prion diseases effective treatments are crucial. Current research has shown that nonpsychoactive cannabidial (CBD) inhibits the accumulation of prion proteins, which give rise to the possibility of an effective treatment. Hopefully, a better understanding of the function of CBD in hindering the aggregation of infectious prions will allow for the development of more treatments that prevent prion diseases [18]. However, several questions remain to be answered, including what effect does Aβ42-oligomer binding have on the functions of PrP^{C}, how do the levels of PrP^{C} compare in the brains of AD patients and age-matched controls, and what is the effect of altering PrP^{C} levels in mouse models of AD. Clearly understanding the molecular and cellular mechanisms involved in the interactions between PrP^{C} and APP/Aβ is crucial to our understanding of AD pathogenesis and warrants urgent further investigation [35].

PrPc may be a key therapeutic target for sporadic AD. As recently suggested that PrPc expression was controlled by AICD (APP intracellular domain) in a γ-secretase dependent manner [36], no evidence for AICD involvement in PrP^{c} expression was found experimentally [37].

Levels of Shadoo (Sho), a protein that resembles the flexibly disordered N-terminal domain of PrP^{C}, were found to be reduced in the brains of mice infected with the RML strain of prions [38], implying that Sho levels may reflect the presence of PrP^{Sc} in the brain. To test this hypothesis, Watts et al. examined levels of Sho during prion infection, and time-course experiments revealed that Sho levels were inversely proportional to levels of protease-resistant PrP^{Sc}. Membrane anchoring and the N-terminal domain of PrP both influenced the inverse relationship between Sho and PrP^{Sc}. Although increased Sho levels had no discernible effect on prion replication in mice, Sho is concluded as the first non-PrP marker specific for prion disease. Additional studies using this paradigm may provide insight into the cellular pathways and systems subverted by PrP^{Sc} during prion disease [39].

During the past decade there have been major advances in our understanding of the fundamental mechanisms of neuronal cell death. Furthermore, not only are caspases important in regulating neuronal cell death during development, they might also mediate cell death in human neurodegenerative diseases. These exciting developments suggest that the targeted inhibition of apoptosis might be effective in the treatment of various neurodegenerative diseases. We do not yet understand exactly how a toxic stimulus, be it trophic factor deprivation, prion protein misfolding and aggregation, Aβ-peptide, triggers the activation of the apoptosis programme in neurons [40].

While host PrP is essential for TSE agent spread and replication, excessive production of all forms of PrP can be inappropriately perpetuated by living cells, even after the initiating infectious agent is eliminated. Host PrP changes can start as a protective innate immune response that ultimately escapes control. A subset of other neurodegenerative and amyloid diseases, including non-transmissible AD, may be initiated by environmental infectious agents that are no longer present. Past and ''cured'' viral infections, some of which may appear to be innocuous, can also lead to a progressive cascade of amyloid changes in membrane proteins such as PrP and APP (the precursor of AD amyloid) that originate as part of an innate protective response against environmental pathogens [41].

Obviously neuronal dysfunction might be initiated before neuronal degeneration, and from a therapeutic point of view a central question is whether the inhibition of neuronal cell death will result in healthy, normally functioning neurons. The answers to these questions and the design of rational therapeutic approaches will require a detailed understanding of how neurons survive and die in the brain [40]. Overall, despite the advances made in prion research, there are still many

questions regarding the proper understanding of nature and function of Prion Proteins under the influence of AD, left unanswered.

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