The pathogenic mechanisms of prion diseases

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Abstract

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of fatal neurodegenerative diseases of humans and animals, including bovine spongiform encephalopathy (BSE) of cattle, scrapie of sheep, and Creutzfeldt–Jakob disease (CJD) of humans. Prion diseases have become an important issue in public health and in the scientific world not only due to the possible relationship between BSE and new variant CJD (nvCJD) but also due to the unique biological features of the infectious agent. Although the nature of the infectious agent and the pathogenic mechanisms of prion diseases are not fully understood, considerable evidence suggests that an abnormal form (PrP\textsuperscii{Sc}) of a host prion protein (PrP\textsuperscii{C}) may compose substantial parts of the infectious agent and that various factors such as oxidative stress and calcium cytotoxicity are associated with the pathogenesis of prion diseases. Here, we briefly review and discuss the pathogenic mechanisms of prion diseases. These advances in understandings of fundamental biology of prion diseases may open the possibilities for the prevention and treatment of these unusual diseases and also suggest applications in more common neurodegenerative disorders such as Alzheimer’s disease (AD) and Parkinson’s disease (PD).  
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1. Introduction

Prions (from proteinaceous infectious) are novel infectious pathogens that cause a group of fatal neurodegenerative disorders termed transmissible spongiform encephalopathies (TSEs) or prion diseases (Prusiner, 1982; Aguzzi and Weissmann, 1997). Since scrapie, an archetype of prion diseases, was found for the first time in sheep a few centuries ago, various forms of prion diseases have been reported in humans such as kuru, Creutzfeldt–Jakob disease (CJD), Gerstmann–
Prion diseases have become an important issue not only in public health due to the possibility of plausible relationship between BSE and new variant CJD (nvCJD) but also in the scientific world due to the unique biological features of the prion agent (Bruce et al., 1997; Hill et al., 1997). Here, we briefly review and discuss current research topics in the area of prion diseases, especially focusing on physiological functions of PrP\textsuperscript{C} and the pathogenic mechanisms of neurodegeneration in prion diseases.

2. The physiological functions of PrP\textsuperscript{C}

The cellular prion protein, PrP\textsuperscript{C}, is a glycoprotein that is found predominantly in CNS, but lower amounts of it are also found in other tissues (Caughey et al., 1988; Bendheim et al., 1992; Manson et al., 1992; Moser et al., 1995). The post-translational modification of PrP\textsuperscript{C} into PrP\textsuperscript{Sc} is a major molecular basis of all prion diseases (Harris, 1999). PrP\textsuperscript{Sc}, the pathogenic form of host PrP, is accumulated in CNS in prion diseases, which is believed to be one of principal causes of neurodegeneration and/or astrocytosis (Lantos, 1992). So far, a number of postulates have been raised for the normal function of PrP\textsuperscript{C}. PrP\textsuperscript{C} is expressed constitutively on the neuronal cell surface by a glycosyl phosphatidylinositol (GPI) anchor, suggesting that PrP\textsuperscript{C} may function as a receptor or adhesion molecules (Stahl et al., 1987). The finding that PrP\textsuperscript{C} is multiply glycosylated indicates that PrP\textsuperscript{C} may be linked to signal transduction pathway (Caughey et al., 1989). It has also been reported that PrP knockout mice developed normally without any noticeable defect, indicating that this protein may not be essential for development and survival (Bu¨eler et al., 1992). PrP knockout mice were both resistant to prion disease and unable to generate new infectious particles (Bu¨eler et al., 1993). Some other investigators found that PrP\textsuperscript{C} is localized in the synapse and that the absence of PrP\textsuperscript{C} may alter synapse formation, suggesting an involvement of PrP\textsuperscript{C} in neurotransmitter systems of CNS (Collinge et al., 1994; Salès et al., 1998). There is increasing evidence that suggests functional roles of PrP\textsuperscript{C} in...
copper metabolism (Brown et al., 1997; Pauly and Harris, 1998). Despite all these postulations, the physiological functions of PrPC have not yet been fully understood.

It is well known that the functions of brain are mediated by catecholamines including dopamine (DA) and norepinephrine and that damage in the catecholaminergic neurotransmitter system has been found in scrapie-infected rodents (Basant et al., 1994; Yun et al., 1998). It was also reported that scrapie agent affects the cholinergic neurotransmitter system in rat pheochromocytoma (PC12) cell lines (Rubenstein et al., 1991). These reports suggest that there may be a relationship between PrPC expression levels and metabolism of neurotransmitter systems. We have demonstrated that the concentration of dihydroxyphenylacetic acid (DOPAC), a metabolite of DA, and monoamine oxidase (MAO) activity, which is involved in oxidative degradation of DA, were significantly increased in the PC12 cell lines overexpressing PrPC (Lee et al., 1999b). These findings suggest that elevation of MAO activity and degradation of DA caused by PrPC overexpression may play a pivotal role in neurodegeneration in prion diseases. Taken together, these findings suggest that PrPC may play a role in the metabolism of neurotransmitters and that alteration of normal PrPC may be associated with neuronal loss in prion diseases.

The lack of appropriate in vitro cellular expression model system of PrPC makes it difficult to examine the functional roles of PrPC. Recently, we developed a recombinant adeno viral system expressing murine PrPC in several human and murine cell lines and also in hippocampal neurons from mouse brain infected with adenoviral PrP (AvPrP-3F4) (manuscript in preparation). The AvPrP-3F4-infected mice did not show any clinical and pathological change. In these studies, we have found that the unglycosylated form of PrPC was predominantly detected in AvPrP-3F4-infected cells and that overexpression of PrPC or accumulation of the unglycosylated PrPC disrupted cellular adhesion to matrix and adjacent cells. These results suggest a possibility of pathogenic mechanisms in prion diseases that the disruption of normal PrPC expression may cause changes in cell adhesion properties and consequently contribute to neurodegeneration. In addition, the established recombinant adeno viral system in this study can be used as a valuable tool to investigate biochemical properties and roles of PrPC.

Recently, it has also been suggested that PrPC may play an important role of protective antioxidant activity (Brown et al., 2001). To investigate whether PrPC can act as an antioxidant, we established neuronal cell lines from Nagasaki PrP knockout mice (Nishida et al., 1997) and examined the role of PrPC under apoptosis and/or oxidative stress conditions (manuscript in preparation). In this study, we found that PrP knockout cell lines were vulnerable to oxidative stress and apoptosis and that this vulnerability was rescued by transfection with PrPC. In addition, the expression levels of Bax and caspase-3 and released cytochrome c were increased in PrP knockout cell lines. These findings strongly suggest that PrPC may play a central role for an effective antioxidant through caspase-dependent apoptotic pathways in mitochondria. Taken together, these results implicate that disruption of normal function of PrPC in prion diseases may reduce the antioxidant capacity of PrPC and then fail to repress caspase-dependent pathway, resulting in neuronal cell death.

3. The mechanisms of neurodegeneration in prion diseases

Neuronal cell loss is thought to be a principal cause of clinical symptoms in several neurodegenerative disorders such as AD, PD and prion diseases. We have focused on understanding the pathogenic mechanisms involved in the progression of prion diseases, especially in the process of neurodegeneration.

3.1. Increased oxidative stress and mitochondrial dysfunction

There is growing evidence that oxidative stress induced by ROS or free radicals play key roles in the pathogenesis of neurodegenerative disorders including prion diseases (Gotz et al., 1994; Beal, 1995). A number of oxidants are produced as
byproducts in the normal aerobic metabolism and particularly at a high rate in neurodegenerative disorders. CNS is especially vulnerable to oxidative stress that has relatively insufficient antioxidants, consumption of high level of oxygen, and large amount of lipid and metals that can produce free radicals (Halliwell and Gutteridge, 1985).

We have reported that the levels of malondialdehyde (MDA) and heme oxygenase-1 (HO-1), which are oxidative stress markers, and the generating rate of free radicals, especially superoxide anion (O$_2^-$), were significantly increased in the brains of scrapie-infected mice (Choi et al., 1998; Lee et al., 2000). In addition, the activity of Cu/Zn-superoxide dismutase (Cu/Zn-SOD), a cytosolic antioxidant enzyme responsible for scavenging ROS, was not affected by scrapie infection, whereas that of Mn-SOD, a mitochondrial enzyme involved in scavenging O$_2^-$, was markedly decreased in scrapie-infected group. We have also found that increased lipid peroxidation and reduced activities of cytochrome c oxidase and ATPase were observed in mitochondria from scrapie-infected animals. Furthermore, structural abnormalities of mitochondria were also found in the hippocampal and cerebral cortical neurons in scrapie-infected rodents (Choi et al., 1998). These results suggest that the mitochondrial damage and increased oxidative stress may play key roles in the pathogenesis of prion diseases.

To further verify whether mitochondrial dysfunction can be associated with the pathogenesis of prion diseases, we analyzed antioxidant systems and calcium levels in the mitochondria of control and scrapie-infected mice (Lee et al., 2000). In the mitochondria of scrapie-infected mice, level of oxidized form of glutathione (GSSG) and calcium content were markedly increased, whereas mitochondrial membrane potential and energy metabolites (ATP/ADP ratio) were decreased. These results suggest that alterations of mitochondrial permeability transition and of energy metabolites due to disturbed mitochondrial respiratory system may result in abnormal calcium accumulation in the mitochondria of scrapie-infected rodents, indicating that mitochondrial dysfunction caused by oxidative damage, abnormal calcium accumulation and altered energy metabolism may contribute to neurodegeneration in prion diseases.

It has been known that phospholipase D (PLD) can be induced by ROS including hydrogen peroxide (H$_2$O$_2$) and that breakdown of phospholipids by PLD can be recognized as an important signaling in CNS (Klein et al., 1995). Recently, we found that the expression level and enzyme activity of PLD1, which is an isozyme of PLD, were significantly increased in the brains of scrapie-infected group, especially in reactive astrocytes of cerebral cortex and hippocampus (Kim et al., 2001). In addition, PLD1 immunoreactivity was co-localized with PrP$^{Sc}$ in reactive astrocytes of scrapie-infected animals. These results suggest that PLD1 activation, which seemed to be caused by PrP$^{Sc}$, may alter the mitochondrial lipid metabolism and in turn, lead to mitochondrial dysfunction in the pathogenesis of prion diseases.

To elucidate the possible mechanism that nitric oxide (NO)-pathway may be involved in the pathogenesis of prion diseases, we examined the levels of NO-related molecules such as inducible nitric oxide synthase (iNOS) and nitrotyrosine in the brains of scrapie-infected mice during the progression of the disease (unpublished data). Accumulation of PrP$^{Sc}$, spongiform encephalopathy, astrocytosis and neuronal loss were observed about 100 days post-inoculation in the brains of scrapie-infected group, in accordance with the increases in the levels of NO, iNOS and nitrotyrosine. These results suggest that the pathologic process of prion diseases may go with or be propagated via NO-pathway. Taken together, these studies support that oxidative stress and mitochondrial dysfunction may contribute to neurodegeneration in prion diseases.

### 3.2. Disturbance of iron metabolism

It has been known that oxidative stress induced by free radicals is closely associated with altered iron metabolism in neurodegenerative disorders such as AD and PD (Gerlach et al., 1994). Iron aggravates oxygen toxicity via a reaction termed iron-catalyzed Harber–Weiss reaction (Halliwell et al., 1992). In the presence of iron, O$_2^-$ and H$_2$O$_2$ can be easily converted to more harmful species...
such as highly reactive hydroxyl radical (OH\(^{\bullet}\)) via Fenton reaction. Thus, these phenomena between iron and oxidative stress in neurodegenerative disorders led us to investigate iron metabolism in scrapie-infected models.

Previously, we have reported that the levels of total and ferric (Fe\(^{3+}\)) iron were significantly increased and that the redox state of iron was change in cerebral cortex, striatum and brainstem by scrapie infection (Kim et al., 2000b). The change of iron redox state in favor of Fe\(^{3+}\) is known to be a condition for iron to participate in the OH\(^{\bullet}\) formation and lipid peroxidation. Therefore, these results indicate that changes in iron content and its redox state as well as accompanied increase of ROS generation may cause oxidative damage to neurons, leading to neurodegeneration in prion diseases.

To further characterize the involvement of altered iron metabolism in the pathogenesis of prion diseases, we examined the expression levels of iron regulatory proteins (IRPs; IRP1 and IRP2), which are known as iron sensing proteins and act as a central part of iron metabolism, and the binding activities between IRPs and iron-response element (IRE) in scrapie-infected rodents (Hur et al., 2001). In this study, we found that the expression levels of IRP1 and IRP2 and their IRE-binding activities were significantly increased in the brains of scrapie-infected mice, especially in reactive astrocytes of hippocampus and of cerebral cortex. We also demonstrated a markedly increased ferritin expression in the regions similar to those of IRPs expression in scrapie-infected group. Ferritin is known as an iron storage protein; therefore, increased ferritin expression in scrapie-infected animals may be involved in the sequestration of free iron pool and decrease of iron-induced oxidative stress, presenting the role of ferritin to protect brain from iron-induced oxidative damage in prion diseases. Taken together, these studies strongly suggest that some protective mechanisms against iron-induced oxidative damage may occur during scrapie infection and that disturbance of iron metabolism and related oxidative stress may contribute directly and/or indirectly to development neurodegeneration in prion diseases.

### 3.3. Alteration of calcium metabolism

Increasing evidence indicates that alteration of calcium and related proteins play important roles in neuronal cell loss of neurodegenerative disorders including prion diseases (Wang and Kelly, 1995; Stabler et al., 1999). The PrP 106-126, a neurotoxic fragment of prion peptides, is known to be involved in the regulation of intracellular calcium level (Florio et al., 1996, 1998).

Recently, as a neuronal calcium-binding protein that interacts with the presenilins, calsenilin has been suggested to be involved in the proteolytic processing of presenilins in AD (Buxbaum et al., 1998; Choi et al., 2001). To elucidate the role of calsenilin in the pathogenic process of prion diseases, we examined the expression level and localization of calsenilin in the brains of scrapie-infected animals as well as in C6 glioma cells. Interestingly, the expression level of calsenilin was significantly increased in some populations of neurons and reactive astrocytes of scrapie-infected mice and in lipopolysaccharide-treated C6 cells (unpublished data). It has been suggested that S100β, an astrocytic calcium-binding protein, is linked to several neuronal diseases (See review of Schafer and Heizmann, 1996) and that it acts on neurons as well as astrocytes to increase intracellular free calcium levels (Barger and Wan Eldik, 1992). We have also reported that the expression level of S100β was significantly increased in the brains of scrapie-infected animals, especially in reactive astrocytes and amyloid plaques (Kim et al., 1994). These results strongly suggest that impaired calcium homeostasis as evidenced by the increased expression of calsenilin and S100β may play a role in neurodegenerative mechanism of prion diseases.

In our following study, calcium/calmodulin-dependent protein kinase II (CaM kinase II) was also markedly elevated in cerebral cortex and hippocampal CA1 region of scrapie-infected mice (Jin et al., 1999). CaM kinase II is an enzyme that is involved in calcium metabolism and plays a pivotal role in the regulation of long-term potentiation (LTP), a form of synaptic plasticity associated with learning and memory. This result indicates that CaM kinase II may be involved in
dysfunction of synaptic transmission and/or LTP via impaired calcium metabolism. Taken together, these studies suggest that disturbance of calcium homeostasis may contribute to progressive neurodegeneration in the pathogenic process of prion diseases.

3.4. Increases of inflammatory cytokines, chemokines and nuclear factor-kappa B (NF-κB) activity

The cellular and molecular aspects of the neuropathology of prion diseases suggest that inflammatory components such as proinflammatory cytokines and complement proteins may play important roles in deteriorating neuronal damage of prion diseases (Kaltschmidt et al., 1993; McGeer and McGeer, 1995; Kordek et al., 1996; Williams et al., 1997; Allen and Tresini, 2000). It has been reported that activated microglia and astrocytes release cytokines and heat shock proteins in the brains of prion diseases; these may be important pathogenic factors in the process of neurodegeneration in prion diseases (Campbell et al., 1994).

It is possible that inflammatory process may be involved in the progressive neurodegeneration in prion diseases. Supporting this hypothesis, the expression of proinflammatory cytokine genes such as interleukin-1α (IL-1α), IL-1β and tumor necrosis factor-α (TNF-α) were markedly increased in scrapie brains (Kim et al., 1999). The activity of NF-κB was increased significantly in scrapie-infected mice, as was NF-κB immunoreactivity, which was most pronounced in hippocampus and thalamus. The immunostaining for NF-κB was especially intense in reactive astrocytes and PrP-amyloid plaques (Kim et al., 1999). In addition, gene expression of IL-6 and iNOS, representative target genes of NF-κB activation, was detected only in scrapie-infected group (Ju et al., 1998). Our recent data demonstrated that gene expression of the chemokine RANTES (regulated on activation normal T cell expressed and secreted) was detected only in hippocampal region of scrapie-infected mice, mainly in reactive astrocytes and in PrP-amyloid plaques and that the expression level of its receptors (i.e. CCR1, CCR3 and CCR5) were markedly elevated in hippocampal region of scrapie-infected group, especially in reactive astrocytes (Lee et al., 2001). RANTES is a proinflammatory peptide that mediates leukocyte migration and activation (Fisher et al., 1995).

It has been reported that the expression level of cyclooxygenase-2 (COX-2), a highly inducible protein and key element that controls the generation of proinflammatory mediators in peripheral tissues, was increased in neurodegenerative disorders such as cerebral ischemia (Nogawa et al., 1997) and AD (Pasinetti and Aisen, 1998). Recently, Walsh et al. (2000) reported the selective upregulation of COX-2 immunoreactivity in glial cells similar to activated microglia in scrapie-infected brain. To examine a possible association of COX-2 with pathogenesis of scrapie infection, we analyzed the expression level and the cellular localization of COX-2 in the brains of scrapie-infected mice (Kim et al., 2000a). In this study, we found that the levels of COX-2 mRNA and protein were increased in scrapie-infected group and that its immunoreactivity was primarily detected in reactive astrocytes. Interestingly, COX-2 expression was co-localized with PrPSc and with NF-κB in scrapie-infected group. These results indicate that upregulation of COX-2 in reactive astrocytes may be related to the accumulation of PrPSc and it may also lead to the progression of prion diseases, possibly by activation of inflammatory responses. From these observations, we propose that PrPSc accumulation in reactive astrocytes may activate NF-κB through increase of ROS production and in turn, alterations of NF-κB-directed gene expression may contribute to both neurodegeneration and inflammatory responses found in prion diseases. Moreover, the facts that NF-κB is one of the transcription factors for HO-1 and that our previous result showing increased expression of HO-1 in reactive astrocytes of scrapie-infected rodents (Choi et al., 2000) further support the correlation between increased oxidative stress, alteration of inflammatory process and activation of NF-κB signaling pathway in the neurodegenerative process of prion diseases.
3.5. Modes of neurodegeneration: apoptosis or necrosis

The mode of cell death, necrosis or apoptosis, in the pathogenic process of prion diseases still remains to be elucidated. Because increased levels of calcium in mitochondria is one of the characteristic features of necrosis, the observation as discussed above that altered calcium metabolism in mitochondria may lead to mitochondrial dysfunction in scrapie-infected animals strongly support the necrotic pathway of neurodegeneration in prion diseases (Lee et al., 1999a; Choi et al., 1998).

It has also been suggested that apoptosis can be a mode of neurodegeneration in several neurodegenerative disorders including prion diseases (Gi-
ese et al., 1995; Lucassen et al., 1995; Thompson, 1995). Supporting this hypothesis, our recent data demonstrated that the expression levels of Bcl-2 (anti-apoptotic protein) and Bax (apoptosis-inducing protein), the regulatory proteins involved in apoptotic pathway, were changed by scrapie infection (Park et al., 2000). In this study, immunoreactivity of Bax was significantly elevated in the neurons of cerebral cortex and hippocampal CA3 region in scrapie-infected group, whereas that of Bcl-2 was markedly reduced in the neuronal populations of similar regions in scrapie-infected brains, indicating that neuronal cell death seen in prion diseases may be regulated by Bcl-2/Bax-mediated apoptotic pathway. As we described in Section 2, the physiological functions of PrP^C, the results of the study using PrP knockout cell lines also strongly support the apoptotic pathway as a mode of neurodegeneration in prion diseases. In summary, these findings suggest that neuronal cell death in prion diseases may employ both apoptotic and necrotic pathways.

4. Conclusion and perspectives

Here, we briefly reviewed and discuss the current research topics of prion diseases based on our research findings, putting emphasis on the pathogenic mechanisms of these diseases. First, we presented several possible functions of PrP^C; involvement in neurotransmitter metabolism, cell adhesion and antioxidant. Secondly, we demonstrated that increased oxidative stress, mitochondrial dysfunction, disturbances in regulation of iron as well as calcium metabolism and alteration of inflammatory process via NF-kB pathway may be closely related to the pathogenic mechanisms of neurodegeneration in prion diseases. These aggravating factors may directly cause neuronal cell death and/or stimulate glial cell such as microglia and astrocytes to produce cytotoxic mediators i.e. proinflammatory cytokines and ROS, and then indirectly give rise to neurodegeneration in prion diseases via both modes of apoptosis and necrosis. Our precision about the pathogenic mechanisms of prion diseases is collectively summarized in Fig. 1.

In spite of recent intensive researches and rapid progresses in the field of prion diseases, a number of important questions remain unanswered in prion diseases; the true nature of the causative agent (virus, virion or prion?), conversion mechanisms of PrP^C to PrP^Sc, the preference of PrP^Sc accumulation in glia and neurons or both, accurate pathogenic mechanisms of neurodegeneration, precise modes of infection, transmission and neuroinvasion, definite physiological functions of PrP^C, and so on. Nevertheless, we are confident that current slow but steady advances in our understanding of the molecular and biochemical mechanisms in the pathogenesis of prion diseases will soon lead to effective strategies for prevention and therapy of prion diseases and other common neurodegenerative disorders such as AD and PD.

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References


