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New Molecular Insights into Cellular Survival and Stress Responses: Neuroprotective Role of Cellular Prion Protein (PrP^C)

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Abstract Knowledge of the physiological function of cellular prion protein has been acquired from prion diseases such as Creutzfeldt–Jakob disease, as well as PRNP knock out and transgenic mice. Recent progress in neurobiology has further delineated the neuroprotective role played by cellular prion protein. In this paper, we review the role of cellular prion protein in cell survival including its antiapoptotic effect on Bax-mediated cell death and its responses to various environmental stresses including oxidative stress, and ischemia. Finally, we discuss the significance of cellular prion protein in different neurodegenerative diseases and the possible development of future therapies.

Keywords Prion · PrPc · Neuroprotection · Oxidative stress · Cell survival · TSE

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Introduction

There are two different conformations of prion protein (PrP): the cellular isoform (PrP^C), and the pathological isoform (PrP^{Sc}). Owing to the immense impact of the relentless and rapidly progressing dementia seen in transmissible spongiform encephalopathy, the physiological role of PrP^C in cells warrants closer investigation [1].

PrP^C, encoded by the PRNP gene [2], is a 30–35 kDa glycoprotein anchored on the cell surface by a glycosylphosphatidylinositol (GPI) moiety, and is expressed abundantly at synapses [3], in neurons [4] and in glial cells [5].

The physiological function of PrP^C remained obscure until several lines of PRNP knockout mice were generated [6]. Although these PrP null mice are healthy throughout their lifespan, with normal development and equal resistance to scrapie infection [6], they were found to be more susceptible to oxidative stress and apoptosis than wild-type mice [7].

In neuronal cell culture, PrP^C elicited neurite outgrowth and neuronal survival through many pathways including the nonreceptor Src-related family member p59(Fyn), PI3 kinase/Akt, cAMP-dependent protein kinase A (PKA), and MAP kinase as well as regulation of Bcl-2 and Bax expression [8].

In anisomycin-induced apoptosis in retinal explants of neonatal mice, PrP^C-binding peptide, which was used to test the function of PrP^C in cell death and later identified as stress-inducible protein 1 (STI-1), activated both cAMP/PKA and Erk pathways and partially prevented cell death in wild-type rodents, but not in PrP null mice. However, the neuroprotective effect of STI-1 was abolished by treatment with phosphatidylinositol-specific phospholipase C, prion protein peptide 106–126, certain antibodies to PrP^C or a PKA inhibitor, but not with a MEK/Erk inhibitor. There-

fore, PrP^C seems to transduce neuroprotective signals through a cAMP-dependent PKA pathway [9].

PrP^C binds to copper and forms a complex, which protects against oxidative stress [10]. As a high-affinitity ligand for laminin, PrP^C participates in neuronal cell trafficking, adhesion, and maintenance. Moreover, these neuroprotective signals emitted by PrP^C against oxidative stress are transduced through a cAMP-dependent PKA pathway, the same as the pathway against apoptosis [11, 12].

In this paper, we review current experimental evidence about PrP^C to address its essential role in cell survival and protective responses to stress of various kinds, in both in vitro and in vivo studies.

Essential Role of PrP^C in Cell Survival

Neuroprotective Role of PrP^C in Doppel-mediated Cell Death

Doppel is a protein of 179-amino acid residues, coded by a gene named PRND, and located near the PRNP locus [13]. Although Doppel and PrP^C are structurally and biochemically related, the function of Doppel is distinct from that of PrP^C [14]. Evidence of the neurotoxicity of Doppel came from PrP null mouse lines, in which Doppel overexpresses and specifically induces Purkinje cell degeneration, resulting in an ataxic phenotype [15]. Removal of PRND can stop the Purkinje cell degeneration process, confirming that the phenotype is caused by Doppel upregulation in the absence of PrP^C, but not the absence of PrP^C itself. These results are evident in Nagasaki, Rmc0, and Zurich mice [16–18].

Furthermore, PrP null mice can be rescued by overexpression of wild-type PrP transgenes, suggesting that cell surface PrP^C can antagonize the toxic effect of Doppel expressed in the central nervous system [19, 20]. Although Doppel neurotoxicity can be counteracted and prevented by PrP^C, Doppel itself is dispensable in prion disease progression and in the generation of PrP^{Sc} [21].

Abnormal regulation of Ca^{2+}-homeostasis may be involved in the ischemia-related neuronal damage not only in female mice with ectopic expression of Doppel, but also in male PrP null mice, and there could be a gender difference in the roles of PrP^C on intracellular Ca^{2+}-homeostasis following transient ischemia [22]. In addition to its toxicity to neuronal cells, Doppel can also affect white matter and cause widespread leukoencephalopathy. The antagonizing oligodendrocyte-specific expression of full-length PrP^C, under the control of the myelin basic protein promoter, successfully repressed leukoencephalopathy and extended survival; however, cerebellar granule cell degeneration remained unchanged. In contrast, neuron-specific PrP^C expression under the control of the neuron-specific enolase (NSE) promoter antagonized cerebellar granule cell degeneration but not leukoencephalopathy [23]. Therefore, the balance between the expression of Doppel and PrP^C is crucial in determining cell survival, involving both neuronal and glial cells, as well as in responding to external stresses.

During Doppel expression, both heme oxygenase 1 and nitric oxide synthase systems are induced, whereas heat shock protein 60 is not. This indicates that Doppel expression might increase oxidative stress and that the response to stress is specific [24].

Neuroprotective Role of PrP^C in Bax-mediated Cell Death

Bax is one of the Bcl-2 family apoptosis-regulating proteins, via which both caspase-dependent and caspase-independent cell death pathways are regulated. Data from transgenic mice of either disruption or expression of specific genes among the Bcl-2 family confirm that the Bcl-2 family stands in a unique and critical position to control neuronal cell survival [25]. The major antiapoptotic family members, Bcl-x(L) and Bcl-2, and the major proapoptotic proteins, Bax and Bak, show distinct temporal and spatial patterns of expression in the developing brain. Bax and Bcl-2 regulate the apoptotic response to neurotrophic factor deprivation. In fact, the absence of proapoptotic Bcl-2 proteins can enhance the toxicity of neuroexcitatory molecules [26].

In scrapie-infected hamster brain, Bcl-2 mRNA was downregulated, whereas Bax mRNA was upregulated compared with that of the controls. The apoptotic process seems to underpin prion diseases by inducing expression imbalances between Bcl-2 and Bax [27], although there is also evidence showing that Bax is unnecessary for neuron death in Bax−/− mice induced by the BSE strain [28]. The increase of Bax protein found in naturally infected scrapie sheep [29] was not observed, however, in murine scrapie [30]. In addition to differences between species, there are also differences between brain areas. In Bax−/− mice, cerebellar granule neuron apoptosis was rescued but not hippocampus and thalamus cell death [28].

Bax overexpression induces cell death of human neurons within 1 day of expression, whereas coexpression of PrP^C with Bax completely abolishes Bax-mediated cell death in neurons [31], although PrP^C does not protect all cell lines against Bax-mediated cell death [32]. The intact structure of PrP^C is needed to abolish Bax-mediated cell death. Deletion of four octapeptide repeats of PrP^C and familial D178N and T183A PrP mutations was shown to completely or partially eliminate the anti-Bax effect of PrP^C [31]. Furthermore, the neuroprotective form of PrP^C would not necessarily be the cell surface GPI-anchored form, as shown by the fact that cytosolic PrP^C remains effective in inhibiting Bax-induced cell death [33].

Significant increase of apoptosis-related proteins including p53, Bax, caspase-3, poly(ADP-ribose) polymerase (PARP) and cytochrome c has been observed in PrP null cells [34]. In addition, Ca^{2+} levels of mitochondria increased, whereas mitochondrial membrane potentials decreased in PrP-null cells. The neuroprotective effect of PrP^C has been further demonstrated by the observation that the vulnerability of PrP null cells to apoptosis could be rescued by the expression of PrP^C [33]. It has been suggested that the antiapoptotic effect of PrP^C takes place in the caspase-dependent apoptotic pathway in mitochondria [34]. Moreover, in a PrP-null neuronal cell line, the apoptotic process or shortened survival duration was attenuated by TNF-alpha [35]. Bcl-2 induction by TNF-alpha was considered to be the main neuroprotective factor.

Another transgenic mice experiment further demonstrated the apoptotic effect of Bax [36]. Transgenic Tg(PG14) mice, which express a mutant prion protein containing 14 octapeptide repeats and present symptoms similar to human familial prion diseases, were crossed with Bax(−/−) mice to produce Tg(PG14)/Bax(−/−) offspring. Cerebellar granule neuron apoptosis in Tg(PG14) was effectively rescued by Bax deletion, implying that these cells die via a Bax-dependent process. However, Bax deletion failed to prevent the rapid degeneration in the molecular layer of the cerebellum and synaptophysin-positive synaptic endings. The age of symptom onset and the duration of illness were not affected in Tg(PG14)/Bax(−/−) mice. Bax-mediated cell death thus plays an important part in degeneration in prion diseases, but does not sufficiently explain the whole process.

PrP^C prevents apoptosis and acts quite specifically on the Bax-dependent pathway. PrP^C cannot prevent Bak-, tBid-, staurosporine-, or thapsigargin-mediated cell death [36]. Bax activation involves Bax conformational change, mitochondrial translocation, cytochrome c release, and caspase activation [36]. The step at which PrP^C inhibition operates has been investigated by experiments in human neurons and in breast carcinoma MCF-7 cells, which showed PrP^C could prevent neither active caspase-mediated cell death nor Bax-mediated cytochrome c release [37]. This implies that PrP^C might exert its antiapoptotic effect by inhibiting Bax conformational change at the initial stage of Bax activation.

Other Neuroprotective Mechanisms

Laminin has been shown to mediate neuronal differentiation through its interaction with cell membrane receptors, such as integrins, the best known receptors related to neurite formation and neuronal migration [38, 39]. More recent research indicates that PrP^C is a true receptor for laminin, responsible for further neuritogenesis dependent on its binding to the γ-1 carboxy-terminal domain of laminin, for which it has an even higher affinity than integrins [40]. This suggests that the PrP^C-laminin interaction is indispensable for neuroplasticity in neuronal development as well as in the regenerative process, although its relation to the pathogenesis of prion diseases remains obscure.

Fyn is one of the Src family tyrosine kinases, and has been found to regulate T cell development [41]. Current evidence suggests that PrP^C might itself act as a signal transduction protein in cell survival and differentiation, as the caveolin-1-dependent coupling of PrP^C to the tyrosine kinase Fyn has been noticed mainly at neurites [42, 43]. PrP^C also interacts directly with the neural cell adhesion molecule (NCAM) and associates with NCAM at the neuronal cell surface, activating Fyn and enhancing neurite outgrowth [44].

Activation of Fyn kinase produces NADPH oxidase-dependent reactive oxygen species signaling, followed by ERK1/2 phosphorylation in neuroprogenitor, hypothalamic, and lymphoid cells. Therefore, PrP^C has a positive effect on neuronal growth and survival through the Fyn-mediated pathway [45].

Essential Role of PrP^C in Response to Environmental Stress

Neuroprotective Role of PrP^C in Oxidative Stress

The Cu/Zn superoxide dismutase (SOD) defect is one of the major mechanisms involved in neurodegenerative diseases such as motor neuron diseases [46]. The activity of Cu/Zn SOD, the fundamental enzyme in reversal of oxidative cell damage, is reduced in PrP null mice. In vitro studies showed that cerebellar cells lacking PrP^C are more sensitive to oxidative stress [47], whereas in another experiment, the levels of total SOD activity were shown to correlate with the level of PrP^C expressed [48], suggesting that PrP^C plays a protective role against oxidative stress.

Increasing levels of PrP^C expression were linked to increased levels of Cu/Zn SOD activity. PrP^C expression may regulate Cu/Zn SOD activity by influencing copper incorporation into the SOD molecule [49]. PrP^C not only alters copper uptake into cells and enhances copper incorporation into SOD, but also acts as a SOD itself [50, 51].

It is known that the octameric-repeat region in the N-terminal region of PrP^C that binds copper and cerebellar cells in PrP null mice is sensitive to copper toxicity and oxidative stress [52]. Only when copper is incorporated into PrP^C during protein folding is SOD activity endowed on the protein. The PrP^C-dependent SOD activity was abolished by deletion of the octapeptide-repeat region involved in copper binding [50]. Thus, PrP^C might exert its neuroprotective effect through chelating copper by the octameric-repeat

region in the N terminus in accordance with the amount of copper it binds [10, 53]. Once the prion protein binds copper, it may act like a SOD, as mentioned earlier [54]. However, if copper-binding of PrP^C is deficient, activity of cytochrome c oxidase will be reduced, resulting in mitochondrial dysfunction, as well as the production of reactive oxygen species (ROS), which in turn triggers mitochondria-mediated apoptotic neurodegeneration [55]. PrP^C is cleaved at the end of the copper-binding octapeptide repeats through the action of ROS, a process termed beta-cleavage. Beta-cleavage is regarded as an early and critical event in the mechanism by which PrP protects cells against oxidative stress. If a construct of PrP lacks the octapeptide repeats, the protein will then fail to undergo ROS-mediated beta-cleavage, as occurs with the two mutant forms of PrP, PG14, and A116V, associated with human prion diseases [56].

The glutathione system contributes to the clearance of hydrogen peroxide by brain cells and thus prevents free radical damage [57]. In PrP-null mice, neuronal glutathione reductase activity is significantly reduced in parallel with increased susceptibility to H_2O_2 toxicity [58]. PrP^C is thus considered to contribute to the glutathione system. Earlier studies have suggested that the PrP toxic peptide PrP106-126 may reduce glutathione reductase activity in neuronal cultures, but the necessity of the presence of PrP^C has not yet been determined [58]. Recent studies show that PrP106-126 can induce the activation of subsets of intracellular kinases (e.g., ERK1/2), early growth response 1 synthesis and caspase-3 activity, all of which are mediated by NADPH-oxidase activity and oxidative stress, even in cells lacking PrP^C [59, 60]. Thus the toxic peptide PrP106-126 might exert its toxic effect in the absence of PrP^C.

Some investigators have suggested that the pathogenesis of prion disease could be caused by imbalances in metal-catalyzed reactions, which result in an alteration of the antioxidant function [61]. Existing evidence is supportive of this hypothesis as a 50% decrease in copper and a tenfold increase in manganese were found in brain tissues of sporadic Creutzfeldt-Jakob disease (sCJD) patients [62]. Multiple changes such as increased levels of nuclear factor NF-κB and manganese SOD, decreased p53 and mitochondrial numbers, and altered melatonin levels were found in PrP-null mice, suggesting the involvement of various kinds of molecules in PrP^C-mediated neuroprotection [63, 64].

In addition to reduced levels of Cu/Zn SOD activity, two other hallmarks of cellular oxidative damage, protein oxidation, and lipid peroxidation are higher in the brain lysates of PrP-null mice than in wild-type mice of the same genetic background [65], indicating that the physiological function of PrP^C is related to cellular antioxidant defenses [66].

Hypothalamic neuronal GT1 cells were employed in experiments to compare the sensitivity to induced oxidative stress between prion-infected and noninfected cells. The results demonstrated a higher sensitivity in the infected cells, which presented as an increased lipid peroxidation and signs of apoptosis associated with a dramatic reduction in the activities of the glutathione-dependent and SOD antioxidant systems [67].

Hyperbaric oxygen has been used as a model for oxidative stress, and activation of Jun N-terminal kinase (JNK) through which PrP^C and Hsp 70 (heat shock protein 70) are regulated, plays a crucial role in protection against this stress. The activated heat shock transcriptional factor 1 phosphorylated by JNK interacted with HSE in the promoter of PrP^C and resulted in increased gene expression [68]. Paraquat toxicity is another example of oxidative stress. Exposure of rabbit kidney epithelial A74 cells to paraquat led to overexpression of PrP^C, along with higher SOD and glutathione peroxidase activities, which in turn significantly reduced paraquat-induced cell toxicity, DNA damage, and malondialdehyde acid levels [69]. Although mitochondrial respiration is a useful parameter for the determination of cellular metabolic rate as it is a major source of ROS, there was no difference in mitochondrial respiration, respiratory control ratio, and membrane potential between PrP-null and wild-type mice [70].

PrP^C might thus be a stress sensor able to initiate, following copper incorporation, a series of signal transduction processes acting on the antioxidant systems to improve cell defenses [71]. Tests in various oxidative stress models, including prion infection, hyperbaric oxygen stress, and paraquat toxicity, illustrate that PrP^C plays an important role in coping with oxidative stress in the nervous system.

Many binding molecules have been studied to clarify the biological functions of PrP^C. A membrane 66 kDa PrP^C-binding protein has been identified as stress-inducible protein 1 (STI-1) [72]. STI-1 is found mainly in the cytoplasm and might be translocated to the plasma membrane as part of a protein complex. PrP^C interacts with STI-1 in a specific high-affinity manner and the interaction sites have been mapped to amino acid 113–128 from PrP^C and 230–245 from STI-1 [72]. Their interaction induced a neuroprotective response against anisomycin-mediated cell death, which was tested in retinal explants from neonatal mice [72]. In addition, STI-1 has been reported to facilitate PrP^C-dependent SOD activation [73]. PrP^C-STI-1 interaction exerted both antiapoptotic and anti-oxidative stress effects, and thus, STI-1 seems to be a molecule that is essential to the neuroprotective effect of PrP^C.

Neuroprotective Role of PrP^C in Cerebral Ischemia

PrP^C is upregulated early in response to focal cerebral ischemia and the extent of upregulation is highly dependent on the severity of focal ischemia or neuronal damage. The early adaptive cellular response of PrP^C to ischemic

brain injury in vitro suggests that it may also be involved in the regulation of ischemia-induced neuronal cell death in vivo [74].

Expression of phospho-Akt (Ser473) was significantly reduced in PrP-null animals compared with wild-type animals early after ischemia and in sham controls [75]. Furthermore, postischemic caspase-3 activation was significantly enhanced in PrP-null mice. The above results show that PrP^C deletion impairs the antiapoptotic phosphatidylinositol 3-kinase/Akt pathway by reducing postischemic phospho-Akt expression, followed by enhanced postischemic caspase-3 activation and aggravated neuronal injury after cerebral ischemia [75]. Loss of PrP^C was also shown elsewhere to render hippocampal neurons susceptible to ischemic insult [22].

Hypoxia-activated transcription factor, phosphorylated by ERK1/2, may in turn interact with the heat shock element in the PrP^C promoter resulting in gene expression of the PRNP gene. Upregulation of PrP^C expression after cerebral ischemia and hypoxia exerts a neuroprotective effect on injured brain tissue [76], suggesting that PrP^C has physiological relevance to cerebral ischemic injury and may be useful as a therapeutic target for the treatment of cerebral ischemia.

PrP^C deficiency can increase infarct size by approximately 200%, whereas transgenic PrP^C restores tissue viability to some extent. The increased activities of ERK-1/-2, STAT-1 and caspase-3 in ischemic brains of PrP-null mice revealed the mechanism underlying the neuroprotective effect of PrP^C against ischemia [77].

Immunoreactive studies showed that PrP^C accumulates within the penumbra of the hypoxic-damaged adult brain, and in situ hybridization analysis suggests an up-regulation of PrP mRNA during hypoxia. Rodents also showed an accumulation of PrP^C in neuronal soma within the penumbra of ischemic lesions [78].

The above results lend support to the notion that PrP^C is upregulated as an immediate response to cerebral ischemic injury. Although its neuroprotective effect has been demonstrated in these animal studies, we are still far from the stage of applying overexpression of PrP^C to patients with cerebral ischemia.

The Neuroprotective Role of PrP^C in Inflammation

Inflammation can increase cell death, and thus efforts have been made to seek antiinflammatory targets. In in vitro studies, macrophages from PrP null mice showed higher rates of phagocytosis than wild-type macrophages. In in vivo studies, the elimination of GPI-anchored proteins from the cell surface of macrophages from wild-type mice rendered these macrophages as efficient as those derived from PrP null mice, suggesting that PrP^C, one of the GPI-anchored proteins, is involved in modulation of phagocytosis [79]. In the scrapie-infected cells, PrP^C is continually released from the cell surface, where it is associated with trapped antigen-antibody complexes [80]. In other words, PrP^C may have an antiinflammatory function and may be able to reduce hyperactive phagocytosis [79].

Neuroprotective Role of PrP^C in Heat Shock and Hypoglycemia

The heat shock response is a well-known example of a cellular adaptive response to elevated temperature, in which a family of proteins, known as heat-shock proteins, is upregulated [81]. Heat-shock stress not only increases levels of Hsp70 mRNA, but also upregulates PrP^C. Two heat-shock elements (HSEs) were found at positions −680 bp (HSE1; GGAACTATTCTTGACATTGCT), and −1,653 bp (HSE2; TGAGAACTCAGGAAG) of the rat PrP (RaPrP) gene promoter [82]. Therefore, through interaction with HSEs on the RaPrP gene under heat shock, PrP^C synthesis is enhanced. This concomitant induction of PrP^C transcription and translation in human NT-2 cells suggests that PrP^C plays a role in the cellular stress response, and it could be categorized as a heat shock protein. However, the protective role of PrP^C against heat shock is not fully understood.

Hypoglycemia caused a concomitant time-dependent and glucose dose-dependent increase in PrP^C and Hsp70 [82]. In addition, under the stress of hypoglycemia, phosphorylated c-JNK protein levels increased along with prolonged duration of stress in a time-dependent manner [83]. After administration of the specific JNK inhibitor SP600125, the upregulation of PrP^C and Hsp70 under hypoglycemic conditions was abolished. The PrP^C expression in response to hypoglycemia is possibly caused by JNK phosphorylation of a heat-shock transcriptional factor, which then interacts with HSE in the promoter of PRNP gene. Although hypoglycemia may upregulate PrP^C, the protective role of PrP^C against hypoglycemia is not fully understood.

The Neuroprotective Role of PrP^C in Epileptogenesis

Animal studies have shown that PrP-null mice were more susceptible than controls to epileptic seizures provoked by various proconvulsive agents, although their lifespan was not affected [84]. This suggests that PrP^C has a protective function or membrane potential stabilizing effect. The reduced hyperpolarization of hippocampal CA1 cells was evident in PrP-null mice in subsequent studies. Furthermore, more granules were found in several areas of hippocampal sections from the PrP null group, including the granule cell layer, the inner molecular layer of the dentate gyrus, and the infrapyramidal region of CA3. The above histopathologic findings resemble abnormal mossy fiber reorganization in certain epilepsies [85].

From a clinical perspective, many patients with refractory mesial temporal lobe epilepsy were found to have the PRNP variant allele Asn171Ser. This rare polymorphism at codon 171 has been associated with hippocampal sclerosis and various cortical malformations, both of which may result in medically intractable epilepsies [86, 87].

Several possibilities have been proposed to further understand the mechanisms by which PrP^C reduces sensitivity to seizures or neuronal excitability, such as disrupted Ca^{2+}-activated potassium channel, abnormal GABA-A inhibition and mossy fiber reorganization in the hippocampus as well as susceptibility to oxidative stress in neocortex [88]. Therefore, PrP^C might be crucial in membrane potential stabilization and the loss of function would increase seizure susceptibility and even result in irreversible histological changes.

PrPC, Neurogenesis and Plasticity

In recent reports, PrP^C has been shown to participate in synapse development [89] and neuritogenesis in embryonic hippocampal neurons [90]. Upregulation of PrP^C was found in the ischemic brain, and overexpression of PrP^C by gene targeting reduced the ischemic damage [65]. Moreover, PrP^C is present on the surface of highly repopulating hematopoietic stem cells [91, 92]. Retrovirus-mediated PrP^C expression on the bone marrow cells of PrP^C knockout mice could rescue the defective hematopoietic engraftment [92]. These results suggest that PrP^C is associated with neurogenesis and plasticity. In fact, it is indisputable that PrP^C correlates strongly with neuritogenesis through interaction with STI-1 [90], and with synaptic plasticity [93]. Interestingly, Steele et al. [94] have demonstrated that expression levels of PrP^C are parallel to the differentiation of multipotent neural precursors into mature neurons. They also found many more proliferating neurons in PrP^C overexpression mice than PrP^C knockout mice [94]. In summary, PrP^C might be one of the major molecules involved in regulating developmental and adult neurogenesis.

PrPC and Glial Cells

Within the central nervous system, PrP^C is expressed not only on neurons, but also other glial cells, including astrocytes [95]. PrP^C expression regulates the uptake of Cu by astrocytes and thus potentially protects neurons from Cu toxicity [96]. Moreover, astrocytes had been noticed to mediate PrP106-126 toxicity. Normally, PrP106-126 is able to induce cortical astrocyte proliferation [97] and is not toxic to cultures containing neurons deficient in PrP^C. However, PrP106-126 was toxic to cerebellar cells derived from PrP-null mice when those cerebellar cells were cocultured with astrocytes [98]. This toxicity was found to be mediated by glutamate. Therefore, gliosis in prion disease may accelerate the neurodegeneration seen in the later stages of the disease. Massive 4-hydroxynouenal accumulation was identified in astrocytes, but not in neurons or microglial cells, implicating an oxidative stress and subsequent lipid peroxidation occurring in astrocytes in prion disease states [99]. The interaction between PrP^C and glial cells is important in neuron cell survival, and further investigation is needed.

Conclusions

In this review, we have described the essential roles of PrP^C in cell survival by discussing the balance between PrP^C neuroprotection and Doppel neurotoxicity, and the inhibition of Bax conformational change and Bax-mediated apoptosis by PrP^C. Moreover, we have looked at the various responses of PrP^C to different stresses, including the PrP^C-enhanced Cu/Zn SOD activity against oxidative stress, PrP^C-STI-1 interaction against apoptosis, and the upregulation of PrP^C expression after cerebral ischemia.

As more and more neuroprotective mechanisms are clarified through studies of PrP^C, we are obtaining to understand more about this prion protein. We may regard the relentless rapidly progressing degenerative phenomena observed in human transmissible spongiform encephalopathy and animal diseases and models with loss-of-function or dysfunction of prion protein in a more positive way.

The compensatory or rescuing effect of PrP^C in PrP-null mice and cerebral ischemic tissues suggests that external addition or genetic upregulation of PrP^C could provide neuronal restoration and regeneration against outer stresses or inner defects. The presence of PrP^C itself could be relevant, on one hand, in inhibiting Doppel expression or other related apoptotic processes, and on the other hand in protection against different insults. PrP^C maintains normal cellular functions, and possibly plays its most important role in neural cells. At one extreme, as PrP^{Sc} shows, neural cells enter an irreversible degenerative process that progresses exponentially to spongiform encephalopathy, whereas at the other extreme, we see that either overexpression of PrP^C through genetic manipulation or external addition of PrP^C can effectively protect neural cells against various stresses, reduce apoptosis, and perhaps even lengthen normal lifespan. Nonetheless, some similar uses of PrP^C have led to toxicity in stably transfected HEK293 cell line and in the PrP^C-inducible Rov9 cells. The proposed mechanism suggests that intracellular PrP^C could convert human cells to proapoptotic phenotype through caspase 3 activation [100, 101].

It would be worth putting more emphasis on research into the PrP^C-laminin and PrP^C-Fyn interaction if neuritogenesis is to be considered as a way to salvage different pathological

degenerations. Recent reports on PrP^C expression in both hematopoietic stem cells and multipotent neural precursors provided us more understanding of neural regeneration by PrP^C. Focal cerebral ischemia of rats is an ideal animal model to testify the regenerative role of PrP^C. Investigation into how the protein could be effectively applied to patients with ischemic stroke, epilepsy, and neurodegenerative disorders is needed. Although this review envisions new therapeutics for neurological disorders in the future, the transformation rate of the manipulated or external prion protein is still unknown, which raises concerns about the safety of any future therapy.

PrP^C is likely to be revealed as an important component in the maintenance of cell survival. Its role may be further clarified when an encoding gene is knocked out or relevant signal pathways are blocked. It is clear from the aforementioned research that PrP^C plays a neuroprotective role in cell survival and exerts various cellular and molecular responses against environmental stresses. We speculate that PrP^C could become an important research target for greater understanding of other neurodegenerative diseases.

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