Roles of Prion Protein in Virus Infections

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Abstract

The normal cellular prion protein, designated PrP^C, is a membrane glycoprotein expressed most abundantly in brains, particularly by neurons, and to a lesser extent in non-neuronal tissues including lungs. Conformational conversion of PrP^C into the amyloidogenic isoform is a key pathogenic event in prion diseases. We recently found that PrP^C has a protective role against infection with influenza A viruses (IAVs) in mice by reducing reactive oxygen species in the lungs after infection with IAVs. The anti-oxidative activity of PrP^C is probably attributable to its function to activate anti-oxidative enzyme Cu/Zn-superoxide dismutase, or SOD1, through regulating Cu content in lungs infected with IAVs. Oxidative stress could play a pivotal role in the pathogenesis of a wide range of viral infections. Here, we introduce our and others' studies on the role of PrP^C in viral infections, and raise the attractive possibility that PrP^C might be a novel target molecule for development of anti-oxidative therapeutics against not only IAV infection but also other viral infections.

Introduction

Viral infection frequently causes oxidative stress by inducing overproduction of reactive species such as reactive oxygen species (ROS) through enzymatic and non-enzymatic mechanisms in host cells (Camini *et al.*, 2017; Li *et al.*, 2017; Peterhans, 1997a; Schwarz, 1996). ROS are chemically reactive molecules containing oxygen, including superoxide, hydrogen peroxide, and hydroxyl radical. The overproduced ROS overly oxidizes proteins, lipids, and DNA, thereby damaging these molecules in host cells eventually contributing to the pathogenesis of virus infection. Cells are also equipped with anti-oxidative mechanisms to balance cellular redox homeostasis. Superoxide dismutase (SOD), catalase, and glutathione peroxidase are major anti-oxidative enzymes (Sgarbanti *et al.*, 2014). Another reactive species, nitric oxide (NO), also contributes to the pathogenesis of virus infections (Perrone *et al.*, 2013; Peterhans, 1997b). NO is produced by NO synthases (NOSs) and converted into the potent oxidative agent nitroperoxide through interaction with oxygen radicals, particularly superoxide (Akaike and Maeda, 2000). Mitigation of oxidative stress in host cells through either interfering with the oxidative mechanisms or enhancing the anti-oxidative mechanisms, or both, can be therapeutically beneficial for viral infections.

The normal cellular prion protein, designated PrP^{C}, is a membrane glycoprotein tethered to the outer cell membrane via a glycosylphosphatidylinositol anchor moiety and expressed most abundantly in brains, particularly by neurons, and to a lesser extent in non-neuronal tissues including hearts, kidneys, and lungs (Oesch *et al.*, 1985; Prusiner, 1998). Conformational conversion of PrP^{C} into the amyloidogenic isoform is a key pathogenic event

in prion diseases, a group of neurodegenerative disorders, which include Creutzfeldt-Jakob disease in humans and scrapie and bovine spongiform encephalopathy in animals (Prusiner, 1998). Several lines of evidence have suggested that PrP^C might have an anti-oxidative function. PrP^C binds to copper (Cu) ions via the histidine residues within the N-terminally located octapeptide repeat (OR) region, which is comprised of 5 tandem repeats of 8 amino acids (Jackson *et al.*, 2001). PrP^C is suggested to regulate anti-oxidative enzymes, such as Cu/Zn-SOD, or SOD1, via transfer of the bound Cu ions to the enzymes (Haigh and Brown, 2006). Other cellular functions, including cell trafficking, cell adhesion, cell differentiation, cell signaling, and cell survival, have been also suggested for PrP^C (Aguzzi *et al.*, 2008).

We recently found that PrP^C has a protective role against infection with influenza A viruses (IAVs) in mice probably through its anti-oxidative function (Chida *et al.*, 2018). There have been also several reports that PrP^C might be involved in protection against different virus infections through different mechanisms (Alais *et al.*, 2012; Baj *et al.*, 2005; Caruso *et al.*, 2009; Nakamura *et al.*, 2003; Nasu-Nishimura *et al.*, 2008; Thackray and Bujdoso, 2002). Here, we introduce our and others' studies on the role of PrP^C in virus infections.

Anti-oxidative treatments against IAV infection in mice

IAV is an enveloped, negative sense, single-stranded RNA virus, causing seasonal epidemics of influenza (Fiore *et al.*, 2008). High morbidity and mortality are observed in infected people, particularly in the young and elderly and those with underlying chronic diseases in lung or

cardiovascular systems (Fiore *et al.*, 2008). Several lines of evidence indicate that ROS plays a pivotal role in the pathogenesis of IAV infection (Akaike *et al.*, 1990; Oda *et al.*, 1989; Tantcheva *et al.*, 2003; Vlahos and Selemidis, 2014). Mice deficient in NOX2, a subunit of the ROS-producing multi-protein complex enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, showed reduced lung injuries after infection with IAV/X-31 (H3N2) and IAV/Puerto Rico/8/34 (H1N1) (hereafter referred to as IAV/PR8) (Vlahos *et al.*, 2011). The inhibitor of another ROS-producing enzyme xanthine oxidase (XO), allopurinol, also reduced mortality of mice infected with IAV/Kumamoto/Y5/67(H2N2) (referred to as IAV/Kumamoto) (Akaike *et al.*, 1990). These results suggest that NADPH oxidase and XO are major ROS-producing enzymes in lungs infected with IAVs, and that reducing the oxidative mechanism could be effective in treatment for IAV infection. On the contrary, it was shown that administration of pyran polymer-conjugated SOD1 successfully reduced the mortality of mice infected with IAV/Kumamoto (Oda *et al.*, 1989). It is thus suggested that SOD1 might be a major anti-oxidative enzyme in IAV infection and that enhancing the anti-oxidative mechanisms could be also therapeutically effective against IAV infection. Treatment with the NOS inhibitor, N^{ω}-monomethyl-L-arginine, successfully reduced the mortality of mice infected with IAV/Kumamoto (Akaike *et al.*, 1996), suggesting that NO could also play an important role in the pathogenesis of IAV infection.

Anti-oxidative role of PrP^{C} in protection against IAV infection

We showed that PrP^C was expressed by alveolar type 1 and 2 epithelial cells (AT1 and AT2 cells) and bronchiolar Clara epithelial cells in mouse lungs (Chida *et al.*, 2018), and that mice devoid of PrP^C (*Prnp*$^{0/0}$) were highly susceptible to intranasal infection with IAV/PR8, A/Aichi/2/68 (H3N2), and A/WSN/33 (H1N1), with markedly elevated mortality, compared to control wild-type (WT) mice (Chida *et al.*, 2018). Infected *Prnp*$^{0/0}$ lungs were severely damaged, with higher infiltration of inflammatory cells, higher levels of inflammatory cytokines and slightly but significantly higher virus titers than control WT lungs (Chida *et al.*, 2018). AT2 and Clara cells succumbed to apoptosis in infected *Prnp*$^{0/0}$ lungs more than in control WT lungs (Chida *et al.*, 2018). In contrast, AT1 cells were not damaged in infected *Prnp*$^{0/0}$ and WT lungs (Chida *et al.*, 2018). This is consistent with IAV/PR8 infection not damaging AT1 cells in C57BL/6 mice (Yamada *et al.*, 2012). These results indicate that PrP^C could have a protective role against lethal infection with IAVs in mice by mitigating lung injuries induced by IAV infection (Fig. 1).

ROS levels were higher in IAV-infected *Prnp*$^{0/0}$ lungs compared to control WT lungs (Chida *et al.*, 2018). In addition, treatment with butylated hydroxyanisole, a ROS scavenger, decreased the mortality of infected *Prnp*$^{0/0}$ mice to that of control WT mice (Chida *et al.*, 2018). These results suggest that PrP^C could play an anti-oxidative role to reduce ROS levels in IAV-infected lungs, thereby providing a protection against lethal infection with IAVs (Fig. 1). In contrast to higher ROS levels in infected *Prnp*$^{0/0}$ lungs, Cu content and SOD1 activity were lower in infected *Prnp*$^{0/0}$ lungs than in control WT mice (Chida *et al.*, 2018). It is thus conceivable that PrP^C might exert its anti-oxidative role through regulation of

the Cu content and SOD1 activity in IAV-infected lungs (Fig. 1). Tg(PrPΔOR)/*Prnp*$^{0/0}$ mice, which express transgenic mouse PrP with a deletion of the Cu-binding OR region on the *Prnp*$^{0/0}$ background (Yoshikawa *et al.*, 2008), also showed lower Cu content, lower SOD1 activity, and higher ROS levels in their lungs and higher mortality after infection with IAV/PR8 (Chida *et al.*, 2018). These results suggest that the Cu-binding OR region plays an important role for PrP^{C} to regulate the Cu content and SOD1 activity and then to exert the anti-oxidative effect in IAV-infected lungs (Fig. 1).

IAVs primarily infect lung epithelial cells, including AT2 and Clara cells, and then cause oxidative stress in them (Liu *et al.*, 2017; Sgarbanti *et al.*, 2014; Short *et al.*, 2014). *Prnp*$^{0/0}$ epithelial cells do not sufficiently combat the oxidative stress due to lack of PrP^{C}, therefore undergoing apoptosis more easily than WT epithelial cells after IAV infection. The higher apoptosis of *Prnp*$^{0/0}$ epithelial cells then provokes higher inflammatory responses leading to higher production of inflammatory cytokines in infected *Prnp*$^{0/0}$ lungs, eventually causing higher mortality of *Prnp*$^{0/0}$ mice after infection with IAVs.

Roles of PrP^{C} in other virus infections

Other groups have also investigated the roles of PrP^{C} in other virus infections (Alais *et al.*, 2012; Baj *et al.*, 2005; Caruso *et al.*, 2009; Nakamura *et al.*, 2003; Nasu-Nishimura *et al.*, 2008; Thackray and Bujdoso, 2002). Higher neuronal apoptosis was reported in the brains of *Prnp*$^{0/0}$ mice than in control WT mice after infection with encephalomyocarditis virus B variant (EMCV-B), with less infiltration of inflammatory cells including microglia in infected

Prnp$^{0/0}$ brains than in control WT brains (Nasu-Nishimura *et al.*, 2008). EMCV-B was similarly replicated in the brains of infected *Prnp*$^{0/0}$ and WT mice (Nasu-Nishimura *et al.*, 2008). These results suggest that PrP^C might be involved in protection of neurons from EMCV-B infection-induced apoptosis possibly through activation of brain inflammatory responses against EMCV-B infection without affecting EMCV-B replication. It was also reported that PrP^C might be involved in protection against latent infection with herpes simplex virus type 1 (HSV-1) (Thackray and Bujdoso, 2002). Mice overexpressing transgenic PrP^C were sensitive to acute infection of HSV-1 strain SC16 in the central and peripheral neuronal tissues, exhibiting higher mortality than control mice (Thackray and Bujdoso, 2002). However, latent infection of the virus in these tissues was significantly suppressed in these mice (Thackray and Bujdoso, 2002). Lower induction of autophagy was reported in *Prnp*$^{0/0}$ astrocytes than in WT astrocytes after infection with HSV-1 strain 17, suggesting that PrP^C might be involved in induction of autophagy in astrocytes after infection with HSV-1 (Korom *et al.*, 2013). However, it remains to be determined whether or not the enhanced acute infection of HSV-1 and the suppressed latent infection of HSV-1 in PrP^C-overexpressing mice can be attributable to the higher induction of autophagy in HSV-1-infected astrocytes. It was also reported that PrP^C inhibited production of human immunodeficiency virus type 1 (HIV-1) in cultured cells transfected with an infectious HIV-1 molecular clone (Alais *et al.*, 2012). PrP^C disturbed translation of the HIV-1 genomic RNA probably through binding to the genomic RNA (Gabus *et al.*, 2001). It has been further reported that PrP^C might be involved

in protection against infection with coxsackievirus B3 (Nakamura *et al.*, 2003), adenovirus 5 (Caruso *et al.*, 2009), and poliovirus-1 (Baj *et al.*, 2005).

Perspectives

We showed that PrP^C has a protective role against lethal infection with IAVs in mice by exerting ant-oxidative activity (Chida *et al.*, 2018). Worldwide spread of IAVs, which are resistant to the currently available anti-influenza agents, has raised great health concerns about pandemics with these resistant IAVs among human populations (Hurt *et al.*, 2009; McKimm-Breschkin *et al.*, 1998; Mishin *et al.*, 2005). The currently available agents such as neuraminidase inhibitors target the molecules encoded by IAVs, promoting the emergence of the IAVs carrying mutations in the genes encoding the targeted molecules and eventually propagating these resistant mutant IAVs among human populations (Hurt *et al.*, 2009; McKimm-Breschkin *et al.*, 1998; Mishin *et al.*, 2005). Therefore, host molecules involved in protection against IAV infection would be plausible targets for development of anti-influenza agents because the agents targeting host molecules are considered not to induce resistant IAVs. Anti-oxidative therapeutics against IAV infection, by targeting the ROS-generating enzymes or by administrating anti-oxidants or anti-oxidant enzymes, has been shown to successfully protect mice from lethal infection with IAVs (Akaike *et al.*, 1990; Oda *et al.*, 1989; Tantcheva *et al.*, 2003; Vlahos and Selemidis, 2014). Our current findings suggest that PrP^C is a new target molecule for anti-oxidative therapeutics against IAV infection. It has been reported that PrP^C protected neurons from anisomycin-induced apoptosis via interaction

with stress-inducible protein 1 (STI1), a STI1-derived peptide, or anti-PrP antibodies (Chiarini *et al.*, 2002; Zanata *et al.*, 2002), and that the interaction with STI1 could be involved in PrP^C-dependent activation of SOD (Sakudo *et al.*, 2005). It is thus interesting to investigate whether these ligands could elicit the anti-oxidative activity of PrP^C and protect against IAV infection and other virus infections, in which oxidative stress plays a pivotal role in the pathogenesis.

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Disclosure Statement

No competing financial interests exist.

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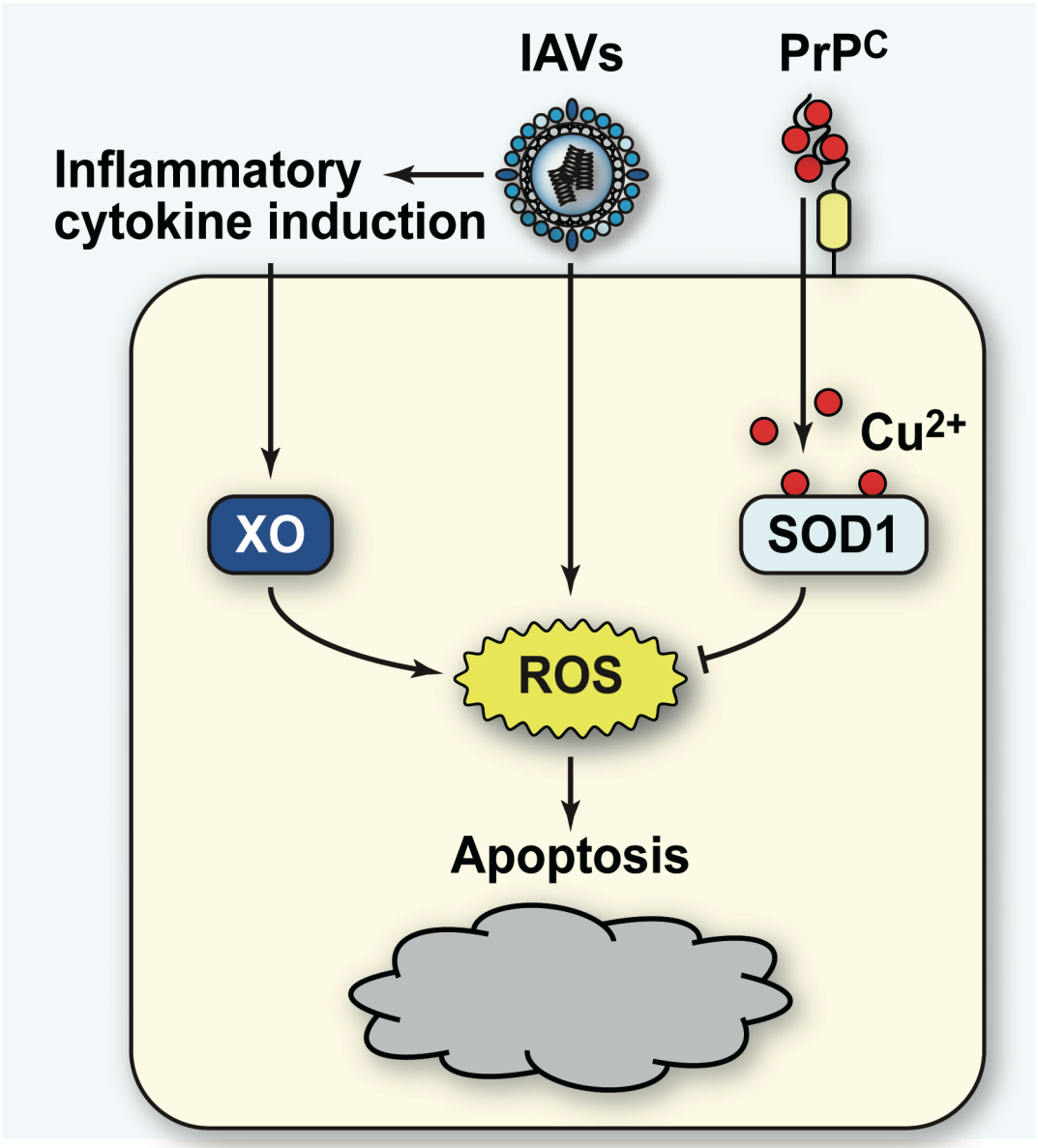
Figure legends

FIG. 1. A possible mechanism for the protective role of PrP^C against IAV infection-induced apoptosis. IAV infection in lung epithelial cells causes overproduction of ROS by directly or indirectly inducing production of inflammatory cytokines, leading to apoptosis in infected epithelial cells. PrP^C could regulate the enzymatic activity of SOD1 by transferring Cu ions, which are bound to the N-terminal OR region, thereby mitigating the burden of ROS in infected cells and eventually protecting the cells from undergoing apoptosis.


IAVs
PrP^C
Inflammatory
cytokine induction
Cu2+
XO
SOD1
ROS
Apoptosis