Cellular prion protein-mediated protection against influenza A virus infection

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Running Head: Prion protein in influenza A virus infection.

Abstract

The cellular prion protein, termed PrP^C, is a glycoprotein abundantly expressed in brains and to a lesser extent in non-neuronal tissues including lungs. It was reported that PrP^C is expressed by lung epithelial cells in mice, and that it may play a protective role against lethal infection with influenza A viruses (IAVs). This may occur by regulating Cu content and superoxide dismutase activity, eventually reducing oxidative stress in infected lungs. Anti-oxidative therapeutics have been demonstrated to protect mice from lethal infection with IAVs. Therefore, PrP^C might be a new target molecule for development of IAV therapeutics. Here, we introduce the anti-viral mechanism of PrP^C against IAV infection and discuss perspectives of PrP^C-targeting therapeutics against IAV infection.

KEYWORDS: prion protein, influenza A virus, reactive oxygen species

Brief overview of prion protein

The normal cellular isoform of prion protein, termed PrP^{C}, is a glycoprotein anchored to the outer cell membrane via a glycosylphosphatidylinositol moiety [1,2]. It expresses most abundantly in brains, particularly by neurons [1,2]. It is also expressed in non-neuronal tissues including heart, lungs, and spleens as well as some in the immune system to a lesser extent [1,2]. Brain accumulation of the abnormally folded, amyloidogenic isoform, PrP^{Sc}, which is generated from PrP^{C} through its conformational conversion, is essential in the pathogenesis of prion diseases [2]. These are a group of neurodegenerative disorders including Creutzfeldt-Jakob disease in humans and scrapie and bovine spongiform encephalopathy in animals [2]. Consistently, mice deficient for PrP^{C} (*Prnp*$^{0/0}$) have been shown to neither accumulate PrP^{Sc} in their brains nor develop prion diseases after inoculation with the infectious agents of these diseases, i.e. prions [3,4,5,6]. However, the exact function of PrP^{C} remains ~~elusive~~ largely unknown.

PrP^{C} has been suggested to be involved in cell protective activities. *Prnp*$^{0/0}$ mice developed enhanced neuronal cell apoptosis after ischemic brain injury [7,8,9]. PrP with a deletion of the N-terminally located octapeptide repeat (OR) region lost the protective activity to rescue *Prnp*$^{0/0}$ neurons from ischemic brain injury [10]. This suggests that PrP^{C} might have an anti-apoptotic role in neurons through the OR region, thereby protecting neurons from ischemia-induced apoptosis. Several lines of evidence indicate that PrP^{C} could bind to Cu ions via histidine residues in the OR region [11,12,13]. Some investigators reported that Cu content and the enzymatic activity of Cu/Zn-dependent superoxide dismutase, or SOD1, are

lower in the brains of *Prnp*$^{0/0}$ mice than in control wild-type (WT) mice [11,14,15]. Therefore, PrP^{C} could play a role in delivery of Cu ions to cuproenzymes such as SOD1, thereby mitigating reactive oxygen species (ROS)-induced oxidative stress. This consequently exerts an anti-apoptotic activity through the OR region. Other neuronal functions have also been suggested for PrP^{C}. Some investigators reported that electrophysiological activities such as GABA receptor-mediated fast transmission and long-term potentiation were impaired in hippocampal slices from *Prnp*$^{0/0}$ mice [16]. This suggests a role for PrP^{C} in learning and memory processes. However, normal GABA receptor-mediated fast transmission and long-term potentiation have been reported in *Prnp*$^{0/0}$ hippocampus by others [17]. PrP^{C} has also been suggested to play a role in long-term survival of cerebellar Purkinje neurons [18], regulation of chemoreceptor numbers at the neuromuscular junction [19], and in controlling circadian activity rhythms and sleep patterns [20]. *Prnp*$^{0/0}$ mice were also reported to be vulnerable to ischemic injuries to their hearts and kidneys [21,22]. This indicates that PrP^{C} could have a protective role against ischemic injuries in non-neuronal tissues as well. PrP^{C} is also expressed in immune cells, including T-lymphocytes, natural killer cells, macrophages, and mast cells [23,24,25]. This suggests a role for PrP^{C} in the immune system.

It was recently reported that PrP^{C} is expressed by lung epithelial cells, including alveolar type 1 (AT1) and 2 (AT2) cells and bronchiolar Clara cells in mice [26], and that PrP^{C} could provide some protection against infection with influenza A viruses (IAVs) via the OR region, by controlling Cu content and SOD1 activity [26]. This eventually reduces oxidative stress in infected lungs [26]. Here we discuss the protective mechanism of PrP^{C} against IAV

infection.

Pathogenic role of ROS in IAV infection

IAVs are enveloped, single-stranded negative-sense RNA viruses, causing seasonal epidemics of influenza. Influenza is an acute respiratory disease with fever, nasal secretions, cough, high temperature, aching joints, and general malaise [27]. IAV infection has been a great public health concern [27,28,29]. Tens of millions of people seasonally suffer from IAV infection. From 250,000 to 500,000 people, particularly the young and elderly and those with underlying chronic lung or cardiovascular diseases, eventually die from influenza or its complications worldwide each year.

The pathogenic mechanism of the IAV infection-induced lung injuries has been extensively studied. It has been suggested that ROS could play a pivotal role in lung injury after infection with IAVs, by causing apoptosis in infected lung epithelial cells [30,31,32,33,34]. ROS are chemically reactive molecules that contain oxygen, including superoxide, hydrogen peroxide, and hydroxyl radical. ROS overproduced in response to IAV infection overly oxidizes proteins, lipids, and DNA [35,36,37,38]. This damages these molecules in host cells eventually contributing to the pathogenesis of IAV infections [35,36,37,38]. Indeed, it has been demonstrated that treatment with the anti-oxidative agent N-acetyl-L-cysteine markedly suppressed ROS in the lungs of mice infected with IAV/WSN/33 (H1N1) (hereafter referred to as IAV/WSN) and decreased mortality of the infected mice [39,40].

Numerous enzymes are involved in generation of ROS. These include mitochondrial electron transport chain complexes, nitric oxide synthase, cytochrome P450 reductases, the nicotinamide adenine dinucleotide phosphate oxidase family (NOXs), and xanthine oxidase (XO) [32]. Mice deficient in NOX2 showed reduced lung injuries after infection with IAV/X-31 (H3N2) and IAV/Puerto Rico/8/34 (H1N1) (referred to as IAV/PR8) [33]. Allopurinol, an inhibitor of XO, also reduced mortality of mice after infection with IAV/Kumamoto/Y5/67(H2N2) (referred to as IAV/Kumamoto) [30]. These results suggest that NOX2 and XO could be major ROS-generating enzymes in IAV infected lungs.

Cells are also equipped with anti-oxidative mechanisms to balance cellular redox homeostasis [32]. The SOD family, which includes SOD1, Mn-dependent SOD (SOD2), and extracellular SOD (SOD3), is one of the major enzyme classes that catalyze ROS detoxification reactions [41]. Infection with IAV/WSN and IAV/chicken/Hubei/327/2004 (H5N1) (referred to as IAV/Hubei) reduced SOD1 expression at mRNA and protein levels in lung epithelial cells [40,42]. SOD1 overexpression was then shown to decrease ROS production in infected lung cells [40,42]. Moreover, it was shown that treatment with SOD1 that is conjugated with pyran polymer successfully decreased the mortality of mice after lethal infection with IAV/Kumamoto [31]. These results indicate that SOD1 may be one of the major anti-oxidative enzymes responding to IAV infection.

Agents such as neuraminidase inhibitors are currently in clinical use. However, worldwide spread of IAVs resistant to these agents has raised health concerns about pandemics caused by these resistant IAVs among human populations [43,44,45]. Currently

available agents target the molecules encoded by IAVs, promoting the emergence of IAVs carrying mutations in the genes encoding the targeted molecules [43,44,45]. These resistant mutant IAVs may eventually propagate among human populations [43,44,45]. Agents targeting host molecules are considered not to induce resistant IAVs. Since NOX2, XO, and SOD1 are host molecules, the agents targeting these molecules might be plausible therapeutics for IAV infection.

Protective activity of PrP^C against IAV infection

It was recently reported that, compared to control WT mice, *Prnp*$^{0/0}$ mice were highly sensitive to intranasal infection with IAV/PR8, A/Aichi/2/68 (H3N2), and IAV/WSN, and showed markedly elevated morbidity and mortality [26]. IAV/PR8-infected *Prnp*$^{0/0}$ lungs were more severely damaged than control WT lungs [26]. Inflammatory cells were more abundantly infiltrated, inflammatory cytokines were more highly produced, and virus titers were slightly but significantly higher in IAV/PR8-infected *Prnp*$^{0/0}$ lungs than in control WT lungs [26]. AT2 and Clara cells in IAV/PR8-infected *Prnp*$^{0/0}$ lungs exhibited higher apoptosis than those in control WT lungs [26]. In contrast, consistent with that AT1 cells in C57BL/6 mice were intact after IAV/PR8 infection [46], AT1 cells were not damaged in IAV/PR8-infected *Prnp*$^{0/0}$ and WT lungs [26]. PrP^C was expressed by AT1 and AT2 cells and bronchiolar Clara cells [26]. These results indicate that PrP^C could provide some protection against lethal infection with IAVs in mice probably by exerting anti-apoptotic activity in lung epithelial cells after IAV infection (Fig. 1). It was also shown that Tg(PrPΔOR)/*Prnp*$^{0/0}$ mice,

which transgenically express mouse PrP with the OR region deleted on the *Prnp*$^{0/0}$ background [47], showed higher mortality after infection with IAV/PR8 [26]. This indicates that the OR region could play an important role for PrP^{C} to provide its protective activity against IAV infection.

The highly pathogenic H5N1 family of avian IAVs also infect neurons, causing apoptotic cell death in infected neurons, eventually developing neurological symptoms [48]. PrP^{C} is expressed in neurons more abundantly than in lung epithelial cells, suggesting that PrP^{C} could also protect neurons from apoptosis after infection with H5N1 avian IAVs. For development of therapeutics against H5N1 avian IAVs, it is worthwhile to investigate whether or not PrP^{C} could provide protection against H5N1 avian IAVs in lung epithelial cells as well as in neurons.

Anti-oxidative activity of PrP^{C} against IAV infection

The OR region is considered to be a site for PrP^{C} to bind to Cu ions and deliver them to SOD1, thereby controlling SOD1 activity, eventually reducing oxidative stress [11,14,15]. Consistent with this, Cu content and SOD1 activity in IAV/PR8-infected *Prnp*$^{0/0}$ lungs were lower than those in control WT lungs [26]. In contrast, ROS levels were higher in IAV/PR8-infected *Prnp*$^{0/0}$ and Tg(PrPΔOR)/*Prnp*$^{0/0}$ lungs than in control WT lungs [26]. Furthermore, butylated hydroxyanisole, a ROS scavenger, decreased the mortality of *Prnp*$^{0/0}$ mice infected with IAV/PR8 to that of control WT mice [26]. These results indicate that the protective activity of PrP^{C} against IAVs infection could be attributable to the anti-oxidative

activity of PrP^C through regulating the content of Cu and consequently the activity of SOD1 in infected lungs via the OR region (Fig. 1). It was also found that XO was more highly expressed in *Prnp*$^{0/0}$ lungs than in control WT lungs after infection with IAV/PR8 [26]. Furthermore, the XO inhibitor allopurinol reduced the mortality of *Prnp*$^{0/0}$ mice infected with IAV/PR8 [26]. These results suggest that the higher ROS production in IAV-infected *Prnp*$^{0/0}$ lungs could also be attributable to the higher expression of XO. The lower SOD1 activity in *Prnp*$^{0/0}$ epithelial cells might fail to efficiently reduce oxidative stress during infection with IAVs, causing higher apoptosis of infected epithelial cells (Fig. 1). This eventually provoked stronger inflammatory responses leading to higher production of inflammatory cytokines in *Prnp*$^{0/0}$ lungs and higher mortality of IAVs-infected *Prnp*$^{0/0}$ mice.

Future perspective

Anti-oxidative treatments have been successful against IAV infection, protecting mice from lethal infection with IAVs [30,31,32,49]. Indeed, the XO inhibitor allopurinol and polymer-conjugated SOD1 reduced mortality of mice after infection with IAV/Kumamoto [30,31]. It was shown that PrP^C could provide some protection against lethal infection with IAVs in mice through its anti-oxidative activity by regulating SOD1 [26], raising the possibility that PrP^C could be a new target molecule for development of therapeutics against IAV infection. However, compared to WT lungs, *Prnp*$^{0/0}$ lungs produced slightly higher titers of IAVs after infection with IAVs [26], indicating that PrP^C might have only marginal effects on IAV replication. Thus, PrP^C-targeting anti-oxidative therapeutics might be more effective

for treatment of IAV infection in combination with other therapeutics suppressing IAV replication.

It has been reported that stress-inducible protein 1 (STI1) and anti-PrP antibodies interacted with PrP^C and protected neurons from apoptosis induced by anisomycin [50,51]. The interaction with STI1 with PrP^C was also shown to activate SOD [52]. Identification of the agents, which interact with PrP^C and then activate SOD in the same way as STI1 and anti-PrP antibodies can, would be very useful for development of a new type of anti-oxidative therapeutic agent in IAV infection. This is currently under investigation in our laboratory. These agents might be safe for clinical use since they are not expected to induce drug-resistant IAVs.

Executive summary

Brief overview of prion protein

- PrP^C is a membrane glycoprotein and expressed most predominantly in brains and to a lesser extent in non-neuronal tissues including lungs.
- PrP^C has been suggested to be involved in various cellular functions.
- It was recently found that PrP^C is expressed by lung epithelial cells and has a protective activity against infection with IAVs by regulating Cu content and SOD1 activity.

Pathogenic role of ROS in IAV infection

- IAVs are enveloped, single-stranded negative-sense RNA viruses, causing great public health concern about seasonal epidemic outbreaks of influenza.

- ROS could have a pivotal role in lung injury after infection with IAVs.
- NOX2 and XO could be major ROS-generating enzymes in IAV infection.
- SOD1 could be one of the major anti-oxidative enzymes responding to IAV infection.
- Anti-oxidative therapeutics targeting NOX2, XO, and SOD1 might be worthwhile to for consideration as a new type of therapeutic agent against IAV infection.

Protective activity of PrP^C against IAV infection

- PrP^C could provide a protection against lethal infection with IAVs in mice.
- The OR region could be important for PrP^C to exert its anti-apoptotic activity in lung epithelial cells after IAV infection.

Anti-oxidative activity of PrP^C against IAV infection

- The protective activity of PrP^C against IAV infection could be attributable to its anti-oxidative activity.
- PrPC could regulate the Cu content and SOD1 activity in infected lungs through the OR region.

Future perspective

- PrP^C could be a new target molecule for development of anti-oxidative therapies against IAV infection.

Financial & competing interests disclosure

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Ethical conduct of research

The authors state that all animal experiments were conducted after the Ethics Committee of Animal Care and Experimentation of Tokushima University approved this study (approval number T27-86).

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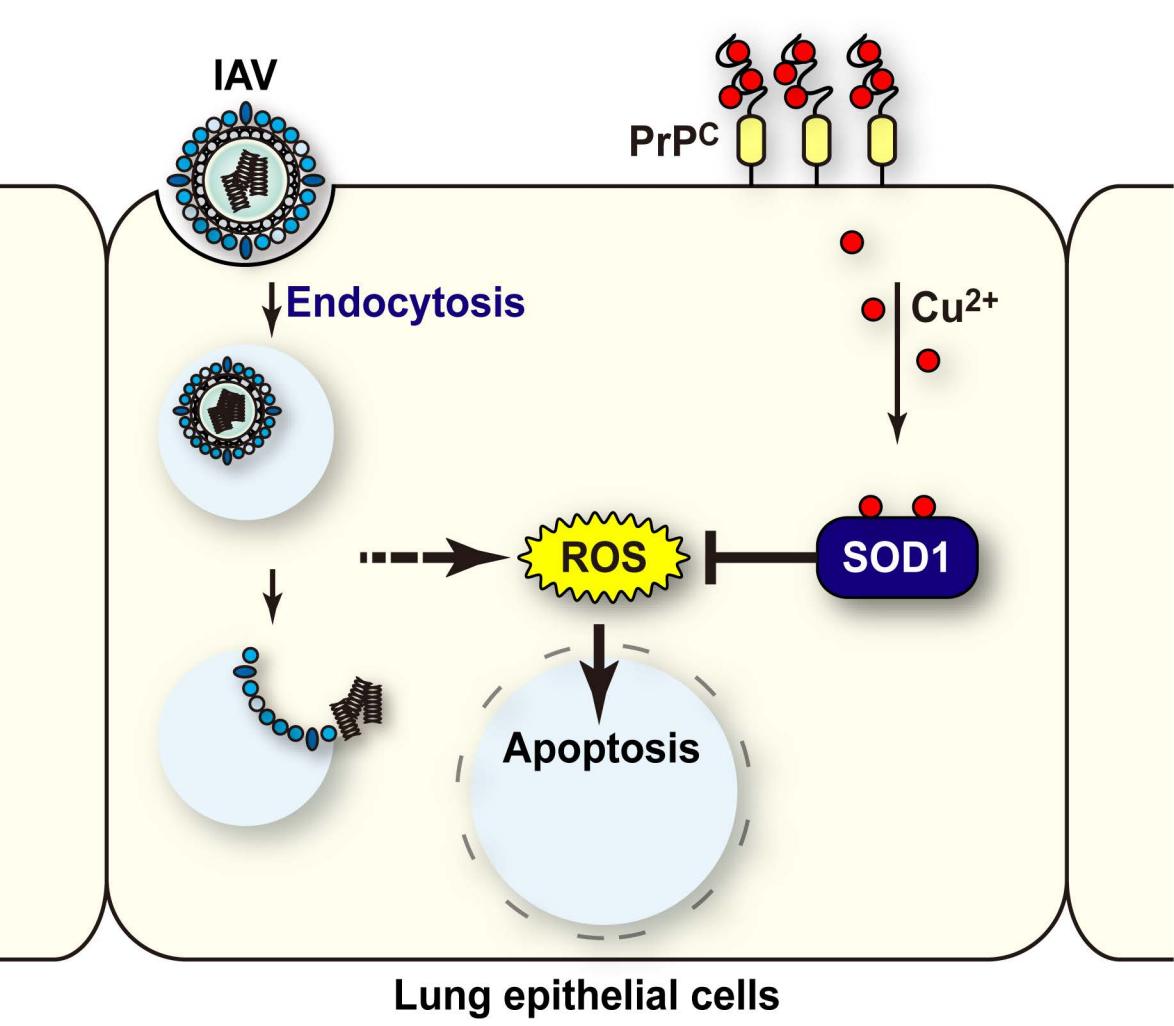
Figure legends

Figure 1. Possible mechanism for the protective role of PrP^C against IAV infection. To establish infection, IAV particles attach lung epithelial cells, enter the cells through an endocytic pathway, and release the viral ribonucleoproteins from the endosome compartments into the cytoplasm. IAV infection leads to overproduction of ROS in lung epithelial cells, causing apoptosis of the cells. PrP^C could play a protective role against IAV infection by reducing ROS levels through activating the anti-oxidative enzyme SOD1. PrP^C binds to Cu ions through the OR region and delivers them to SOD1, thereby activating it. The activated SOD1 then reduces ROS levels in IAV-infected epithelial cells and eventually inhibits the cell from undergoing apoptosis.


IAV
PrPC
Endocytosis
Cu2+
ROS
SOD1
Apoptosis
Lung epithelial cells