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The association between prion proteins and $A\beta_{1-42}$ oligomers in cytotoxicity and apoptosis

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ABSTRACT

Misfolding of prion protein (PrP to PrPSc) can cause neurodegenerative prion diseases. As a glycosylphosphatidylinositol (GPI)-anchored membrane protein, the normal form of PrP (PrPC) can function as a receptor for ligands in the extracellular space. PrPC was suggested to be involved in memory, synaptic neuronal communication, and anti-oxidation as a neuroprotective agent. The recently identified interaction between PrPC and $A\beta_{1-42}$ oligomers suggested another role for PrP as a receptor for $A\beta_{1-42}$ oligomers, thereby influencing cytotoxicity and apoptosis.

Here, the association between PrPC and $A\beta_{1-42}$ oligomers was investigated by visualizing protein localization in neuronal cells by immunocytochemistry. $A\beta_{1-42}$ oligomer-induced cytotoxicity was tested in respective expressions of PrPC by using mouse neuroblastoma-2a (N2a) cells, the prion protein overexpressed cells (L2-2B1), and a Prnp-null mouse hippocampal cell line (HpL 3–4). Moreover, apoptotic proteins such as caspase-8 were used to assess the effect of PrPC on $A\beta_{1-42}$ oligomer-mediated apoptosis. In L2-2B1 and HpL 3–4 cells, the difference in the cytotoxicity of $A\beta_{1-42}$ oligomers could be clearly distinguished. In addition, $A\beta_{1-42}$ oligomers induced mitochondria dysfunction, reactive oxygen species (ROS) generation, and calcium influx PrPC-dependently. Apoptosis, related to mitochondria dysfunction, was further investigated to determine the cytotoxic pathway; the results suggest that PrPC could be involved in both the intrinsic and extrinsic apoptotic pathways. Finally, cells with abundant PrPC expression seemed to be more susceptible to $A\beta_{1-42}$ oligomer toxicity, suggesting the importance of the level of PrPC expression in the induction of apoptosis.

1. Introduction

Prion diseases, i.e., Creutzfeldt–Jakobdisease (CJD) in humans; bovine spongiform encephalopathy (BSE) in cattle; scrapie in sheep, hamsters, and mice; as well as chronic wasting diseases in cervids, are neurodegenerative disorders [1]. The general pathogenesis of prion diseases involves the structural conversion from the normal cellular prion protein (PrPC) to the infectious form of PrP (PrPSc). Because PrPSc causes the PrPC to become functionally inactive, the loss of normal function of PrPC was hypothesized to be a cause of neuronal cell death [2]. PrPC was suggested to have an antioxidant role through its participation in neuroprotective signaling on the cell surface [3]. PrPC is exposed to the extracellular matrix. Recently, several ligands for PrPC were suggested to trigger neuronal dysregulation [4]. Among them, beta-amyloid ($A\beta_{1-42}$) oligomers, causing synaptic dysfunction, were shown to have a high affinity for PrPC [5,6]. Since $A\beta_{1-42}$ oligomers are a major component of senile plaques in Alzheimer's disease (AD), the cytotoxic mechanism from the interaction between $A\beta_{1-42}$ oligomers and PrPC were investigated *in vitro* in the present study. $A\beta_{1-42}$ oligomers could cause damage to neuronal cells through various apoptosis pathways by causing mitochondrial dysfunction and calcium influx; PrPC may provide the necessary pathway for $A\beta_{1-42}$ oligomer-mediated neuronal cell death [7]. Hence, the cytotoxicity from $A\beta_{1-42}$ oligomers was investigated using PrPC-overexpressing, normal, and PrPC-deficient cell lines to determine whether oligomer-induced cytotoxicity depended on PrPC expression levels. We also investigated different apoptotic pathways. Our study may provide a basis to understand common symptomatic pathologies in CJD and AD.

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2. Materials and methods

2.1. Cell lines

The cell lines used were mouse neuroblastoma-2a (N2a) from the ATCC, USA; L2-2B1, which is overexpressed cell line of N2a transfected with the full length of mouse PrP, established by Dr. Kim of the Korean National Institute of Health; and the Prnp-null mouse hippocampal cell line (HpL 3–4), which was a generous gift from Dr. Onodera at Tokyo University.

2.2. Preparation of $A\beta_{1-42}$ oligomers

Aggregated stock solutions of $A\beta_{1-42}$ oligomers were prepared in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Sigma) according to a previously described method [8].

2.3. Western blot

Tricine–PAGE was used for species separation of $A\beta_{1-42}$ oligomers. $A\beta_{1-42}$ oligomers (20 μM) were electrophoresed on a 16.5% Tris–Tricine gel, which was transferred to a membrane using the i-Blot system (Invitrogen). The membrane was blocked, then incubated overnight at 4 °C with the beta-amyloid monoclonal antibody (4G8, Covance), followed by incubation with anti-rabbit horseradish peroxidase (HRP)-linked secondary antibody. The bands were visualized by enhanced chemiluminescence (ECL) detection reagents (Pierce) and imaged on film (Agfa). For identification of PrPC concentration in the cells, SDS–PAGE was applied. *DC*™ protein Assay kit (Bio-Rad, USA) for total protein quantification in the cells was used. The bands of PrPC and β-actin were scanned and quantified using densitometric software (Quantity One, Bio-Rad).

2.4. Observation of the oligomers by AFM

Morphological images of $A\beta_{1-42}$ oligomers were investigated using Atomic Force Microscopy (AFM, Dimension 3100; Veeco). Ten microlitres of distilled water and 5 μM of $A\beta_{1-42}$ monomer and oligomers were each placed on a glass slide. After incubation in a dry oven, the samples were measured. AFM was operated in tapping mode (resonant frequency, 280–290 kHz) in air at room temperature with a scanning rate of 1–2 Hz. The sample roughness was determined using the software provided by Veeco.

2.5. Measurement of cell viability

The viability of N2a, L2-2B1, and HpL 3–4 cells was measured using the MTT assay. The cells were seeded in a 96-well plate. After incubation for 24 h, the medium was replaced with FBS-free containing $A\beta_{1-42}$ oligomers at 0.1, 1, 10 and 20 μM. Control experiments were performed in which $A\beta_{1-42}$ oligomer was not treated. After the cells were incubated for 24 h, 10 μL of MTT solution (5 mg/mL in filtered with PBS) was added and then incubated for 4 h. The remaining formazan crystals were solubilized with DMSO. After gentle shaking, the absorbance at 590 nm was read with an ELISA plate reader (Bio-Rad). The results are expressed as the percentage of MTT reduced relative to the control samples, assuming the absorbance of the control cells was 100%.

2.6. Measurement of intracellular ROS

Intracellular reactive oxygen species (ROS) formation was evaluated using 2′,7′-dichlorofluorescein diacetate (DCFH-DA). The cells were treated as described above. After incubation, the remaining cells were exposed to 100 μM of DCFH-DA for 1 h. The fluorescence was measured with a plate reader (VICTOR3; Perkin-Elmer) with excitation and emission wavelengths of 485 and 535 nm, respectively [9].

2.7. Measurement of cytosolic free Ca^{2+}

Cytosolic $[Ca^{2+}]_I$ levels were measured with the ratiometric fluorescent indicator, indo-1 AM (Invitrogen). The cells were treated as described above. After inducing toxicity, the cells were loaded with 3 μM of indo-1 AM for 1 h. Bound fluorescence was recorded at 400 nm (F_{400}), and unbound fluorescence was measured at 475 nm (F_{475}) with excitation at 355 nm. The change in $[Ca^{2+}]_I$ was expressed as a change in the ratio $R = F_{400}/F_{475}$.

2.8. Immunocytochemistry: discrimination of apoptotic cells

The discrimination of apoptotic cells from live cells after treatment with 10 μM of $A\beta_{1-42}$ oligomers was performed using the Apoptotic/Necrotic/Healthy cells detection kit (PK-CA707-30018, PromoKine). 50 μM of Ionomycin (I9657, Sigma) was used as the negative control. The procedure was performed according to the manufacturer's instructions. Fluorescent images were observed with an Axio Scope 2 microscope (Carl Zeiss).

2.9. Immunocytochemistry: detection of co-localization of PrPC and $A\beta_{1-42}$ oligomers

The cells were loaded on Chamber Slide (LAB-TEK®, Nunc) with 10 μM of $A\beta_{1-42}$ oligomers. It was incubated and fixed by adding 4% paraformaldehyde in PBS for 30 min at room temperature. After washing, the cells were blocked with 5% BSA [2 mg/mL] for 1 h, incubated with the working concentration of primary antibodies (1:1000; mouse-specific prion protein antibody [7D9] and rabbit-specific anti-amyloid oligomer [AB9234]) at 4 °C for 4 h. After washing, the cells were treated with anti-mouse IgG FITC-conjugated and anti-rabbit IgG rhodamine-conjugated (AP307R) secondary antibodies for 1 h. Confocal microscopy was performed on a LSM 710 laser scanning microscopy (Carl Zeiss).

2.10. Quantification assay

Caspase 3/7 activity in the cells was quantified by the ApoTox-Glo™ Triplex Assay kit (Promega). The quantitative analysis of caspase-8 activity was performed with the Caspase-8 Colorimetric Assay Kit (PromoKine)).

2.11. Statistical analysis

Each experiment was repeated a minimum of three times. The one-way ANOVA with the Tukey–Kramer procedure was used. Differences were considered as significant at $P < 0.05$.

3. Results

3.1. PrPC expression in cells

Cells with respective expression rates of PrPC were characterized (Fig. 1A). Western blots revealed di-glycosylated (32–34 kDa), mono-glycosylated (24–28 kDa), unglycosylated (17–20 kDa) in total protein (40 μM/mL), quantified by the Dc protein assay. Higher expression of PrPC was observed in L2-2B1 cells than in N2a cells, which was the parental cell line of L2-2B1. HpL 3–4 cells did not show any PrPC expression. (Fig. 1B).

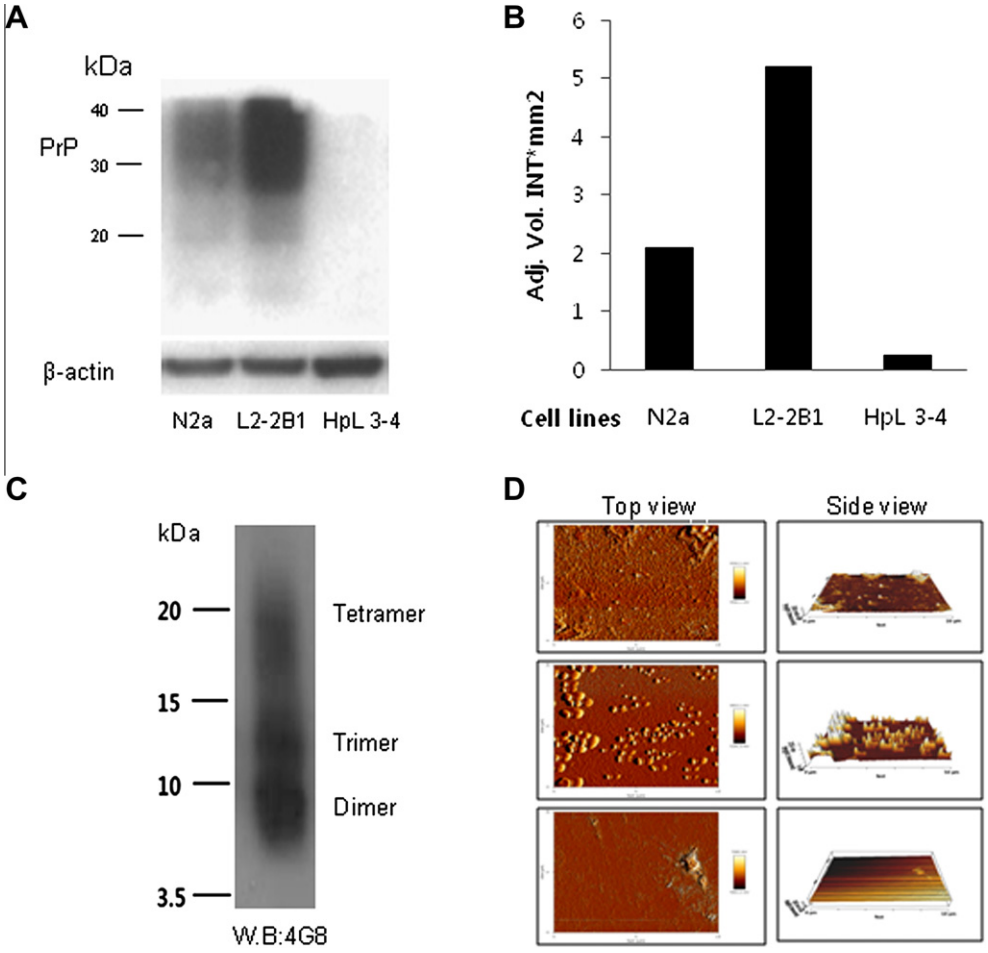

Fig. 1. Certification of PrPC and $A\beta_{1-42}$ oligomers (A) Western blot for PrPC detection in N2a, L2-2B1, and HpL 3–4 cells. The band intensity was showed as the volume intensity by mm^2 relative to the β-actin (B). (C) Western blots for 10 μM of synthetic $A\beta_{1-42}$ oligomer species by using 4G8 antibody. The $A\beta_{1-42}$ oligomers showed bands around 9 kDa (dimer), 13 kDa (trimer), and 18 kDa (tetramer). (D) AFM topographical images of monomeric and oligomeric $A\beta_{1-42}$ and distilled water (as control). The left side shows the original images of AFM, and the right side shows the three-dimensional images rendered by the software. Bar: 1 μm.

3.2. Characterization of $A\beta_{1-42}$ oligomers

Western blot analysis was used to characterize the $A\beta_{1-42}$ oligomers that had a MW range from 9 to 18 kDa (Fig. 1C), which is consistent with low-MW oligomer products [10]. The concentration of dimers, trimers, and tetramers were higher than other oligomeric species. Smears between the dimers and tetramers were consistent with interconverting assemblies. The sizes of the synthetic peptide were confirmed topologically by AFM. $A\beta_{1-42}$ proteins in monomer and oligomer conformations were determined as 3.9 and 13.14 nm, respectively (Fig. 1D), which are consistent with previous reports [11]. A size of 13.14 nm for oligomers translates to 3–4 mers. The sizes were calculated by subtracting the value of distilled water (0.501 pm). Distilled water would not have any roughness, but interestingly, oligomers had much more roughness than monomers (Fig. 1D, side view).

3.3. Changes of cell viability, intracellular ROS and Ca^{2+} in the presence of $A\beta_{1-42}$ oligomers

The cells treated with $A\beta_{1-42}$ oligomers showed a dose-dependent decrease in their viability (Fig. 2A). Although HpL 3–4 cells had a higher viability than other cells, a minor toxic effect could be attributed to the absence of PrPC when $A\beta_{1-42}$ oligomers caused cytotoxicity. L2-2B1 cells had the lowest viability among all cells, which suggests that the overexpressed PrPC could interact with the $A\beta_{1-42}$ oligomers causing cytotoxicity. All cells treated with $A\beta_{1-42}$ oligomers had significantly elevated fluorescence values indicating intracellular ROS production, compared with the untreated cells (named as buffer; Fig. 2B). Fluorescence from L2-2B1 cells was increased markedly upon addition of $A\beta_{1-42}$ oligomers. Also, ROS production in N2a cells was higher than in HpL 3–4 cells after the addition of $A\beta_{1-42}$ oligomers. Increased intracellular ROS generation from mitochondria by $A\beta_{1-42}$ oligomers correlated with the increased expression of PrPC.

$A\beta_{1-42}$ oligomer treatment seemed to impair Ca^{2+} homeostasis, causing an influx of Ca^{2+} into the cytosol (Fig. 2C). Cytosolic calcium levels were significantly higher in L2-2B1 cells treated with $A\beta_{1-42}$ oligomer (10 μM) compared with those treated with 0.1 and 1 μM $A\beta_{1-42}$ oligomers. Concentration of $A\beta_{1-42}$ oligomers over 10 μM appeared to reach peak saturation, and then decline. The changes observed with treatment of them in HpL 3–4 and N2a cells were almost same.

3.4. PrPC-dependent apoptosis

The cytotoxicity from $A\beta_{1-42}$ oligomers in the presence and absence of PrPC was investigated by quantifying apoptosis using a three-colored fluorescence microscopy assay (Fig. 3A). The Hoechst 33342 staining (blue color) was used as a control to stain the nuclei of all cells (live, apoptotic, and necrotic). Apoptotic cells produce a bright green fluorescence in the presence of Annexin V, while

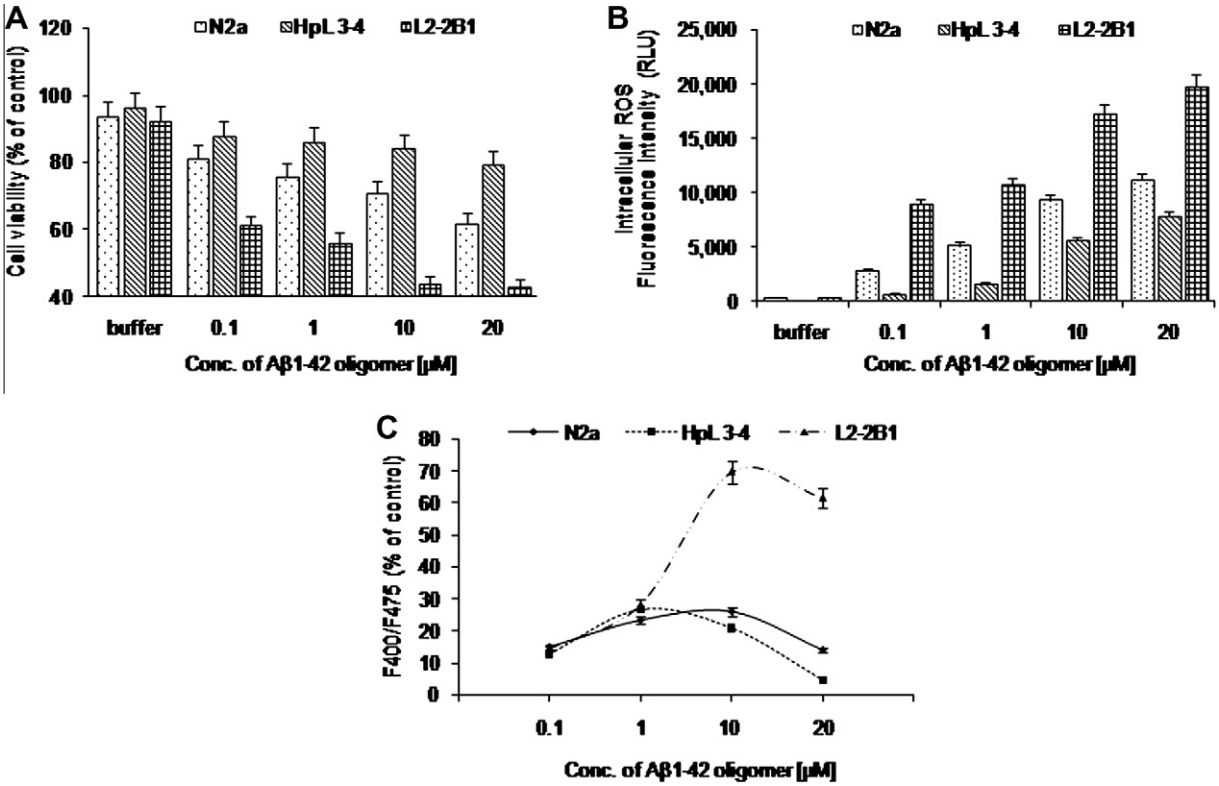

Fig. 2. Cytotoxic effect of $A\beta_{1-42}$ oligomers. The cells were treated with various doses of $A\beta_{1-42}$ oligomers. MTT assay showed in (A). Measurement of ROS with DCFH-DA (B). The effect of $A\beta_{1-42}$ oligomers on basal $[Ca^{2+}]_i$ was observed (C). *In vitro* assays showed L2-2B1 cells were more susceptible to cytotoxicity of $A\beta_{1-42}$ oligomers. These data were subtracted from untreated control cells and each value represents the mean ± S.D. $P < 0.05$ compared with untreated control cells.

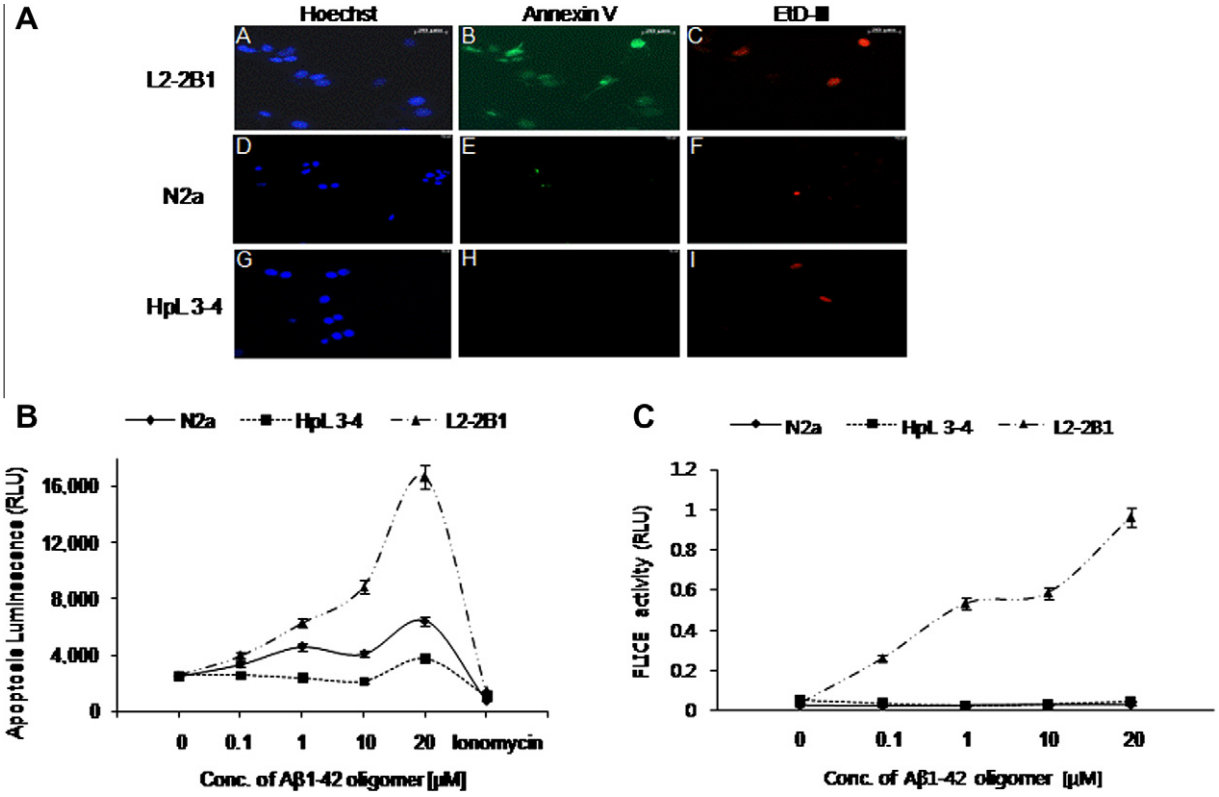

Fig. 3. Confirmation of apoptosis in each cell line (A) Localization of Hoechst 33342, Annexin V, and EtD-III dyes were monitored using a microscope with fluorescent filters. (B) Caspase-3/7 activity during apoptosis was determined. Cells treated with ionomycin (50 μM) were not apoptotic. (C) Caspase-8 (FLICE) activity was determined. (B) and (C) were subtracted from untreated control cells and each value represents the mean ± S.D. $P < 0.05$ compared with untreated control cells. Bar: 20 μm.

necrotic cells produce a red fluorescence in the presence of ethidiumhomodimerIII (EtD-III). After the treatment of $A\beta_{1-42}$ oligomers, L2-2B1 cells displayed bright green and blue fluorescence, indicating that they were undergoing apoptosis; none of the L2-2B1 cells emitted only blue fluorescence. There were some N2a cells undergoing apoptosis. The bright green fluorescence of HpL 3–4 cells was not seen at all, but there were any necrosis in any of the cells. These results suggest that $A\beta_{1-42}$ oligomers initiate apoptosis in neuronal cells when PrPC was abundance. Increased caspase-3/7 activity from $A\beta_{1-42}$ oligomer-dependent apoptosis was also observed, particularly in L2-2B1 cells (Fig. 3B). A little elevation in caspase 3/7 activity was observed in the N2a cells, which is consistent with our cytotoxicity data shown in Fig. 3A as well as HpL 3–4 cells. When all cells were only treated with ionomycin (50 μM), a well-known antibiotic causing necrosis, apoptosis did not occur.

Cleaved caspase-8 or FADD-like interleukin-1 beta-converting enzyme (FLICE) could activate caspases in the downstream of extrinsic apoptosis pathway that is independent of the mitochondrion induced apoptosis (i.e., intrinsic). When caspase-8 levels were compared in the N2a, HpL 3–4, and L2-2B1 cells with or without $A\beta_{1-42}$ oligomer (Fig. 3C), they were found to increase sharply in L2-2B1 cells. A little change in caspase-8 levels were observed in the N2a cells with the treatment of $A\beta_{1-42}$ oligomers at 20 μM, while no changes were observed in the HpL 3–4 cells. Based on these data, we conclude that caspase-8 activation was dependent on PrPC, where $A\beta_{1-42}$ oligomers induce the FLICE-dependent extrinsic apoptosis pathway facilitated by PrPC.

3.5. Co-localization of $A\beta_{1-42}$ oligomers and PrPC on the cell surface

To elucidate the PrPC-dependent effects on cytotoxicity and apoptosis, we performed an immunoreaction to observe the co-localization of PrPC and $A\beta_{1-42}$ oligomers by using fluorescently labeled antibodies (Fig. 4). L2-2B1 cells treated with $A\beta_{1-42}$ oligomers were probed with anti-PrPC and anti-$A\beta_{1-42}$ oligomer antibodies. We hypothesized that if $A\beta_{1-42}$ oligomers and PrPC interact with each other, they would at least form a transient complex in the same region of the cell during the induction of apoptosis. The data in Fig. 4 indicate that the green fluorescence intensity for PrPC was evenly distributed throughout the cells (Fig. 4B). The red fluorescence intensity (Fig. 4C) for $A\beta_{1-42}$ oligomers revealed $A\beta_{1-42}$ clusters, and the high levels of adjacent fluorescence intensity seemed to be located on the same surface near PrPC, where the co-localization could be observed from a merge of the red and green fluorescence (Fig. 4D). Initially, the oligomers seemed to be anchored to the cell surface and did not penetrate into the cytosol, based on the Z-stack data. Even if $A\beta_{1-42}$ oligomers bound to a few membrane proteins throughout the cell surface, high adjacent fluorescence intensity was observed in L2-2B1 cells. Our data was consistent with previous result [5]. There results suggest a possible role of PrPC as a cofactor of $A\beta_{1-42}$ oligomers in mediating subsequent cytotoxicity and apoptosis.

4. Discussion

We investigated neuronal toxicity from treatment with $A\beta_{1-42}$ oligomers in the relative expression of PrPC. Overexpressing a PrPC may cause an ER stress response due to overloading this with ectopic expressed proteins. However, our results revealed the elevated PrPC in L2-2B1 cells is not unglycosylated, but glycosylated PrPC, which is entopic (Fig. 1A).

The formation of low MW $A\beta_{1-42}$ oligomers was analyzed with an immunoassay and AFM (Fig. 1C and D). Since mitochondrial dysfunction is an indicator of cytotoxicity, two cytotoxicity tests, i.e., MTT and DCF assays, were performed to examine cell viability and mitochondrion-induced ROS release, respectively [12]. Increased DCF from the addition of $A\beta_{1-42}$ oligomers indicated the generation and translocation of ROS into the cytosol due to mitochondrial dysfunction. When the role of PrPC in facilitating $A\beta_{1-42}$ oligomer-induced cytotoxicity was investigated, L2-2B1 cells, which overexpress PrPC, were more susceptible to cytotoxicity in the presence of $A\beta_{1-42}$ oligomers, compared to HpL 3–4 cells that lack PrPC. These data suggest the involvement of PrPC in $A\beta_{1-42}$ oligomer-induced cytotoxicity, particularly with the induction of mitochondrial dysfunction. Hence, a direct correlation from the addition of $A\beta_{1-42}$ oligomers was observed in cells with highly expressed PrPC with increased production of intracellular ROS from mitochondria. Here, the L2-2B1 cell line showed a clear difference in cytotoxicity in the presence of $A\beta_{1-42}$ oligomers, where the difference in N2a and HpL 3–4 cells was not evident. In MTT assay, L2-2B1 cells without $A\beta_{1-42}$ oligomers were also induced the toxicity. According to Paitel et al., overexpression of PrPC could trigger caspase-3 activation [13]. It could be considered L2-2B1 cells lead to caspase cascade itself. However, the self toxicity was insignificant. Our results showed $A\beta_{1-42}$ oligomers dependent cytotoxicity is far intervally occurred than self toxicity of PrPC overexpression.

We investigated the role of mitochondria in $A\beta_{1-42}$ oligomer-induced apoptosis, which would indicate the initiation of the mitochondrion-dependent intrinsic apoptotic pathway. Mitochondria are influenced by Ca^{2+} imbalance in the initial apoptotic stages. Hence, the detection of Ca^{2+} influx from the extracellular matrix could be used to assess the cytotoxicity of $A\beta_{1-42}$ oligomers. $A\beta_{1-42}$ oligomers have been shown to quickly activate the existing calcium channels and receptors [14]. It has also been reported that $A\beta_{1-42}$ oligomers may form calcium-permeable pores on the membrane, creating direct influx of calcium into the cytosol [15]. In addition, PrPC has also been reported to be involved with

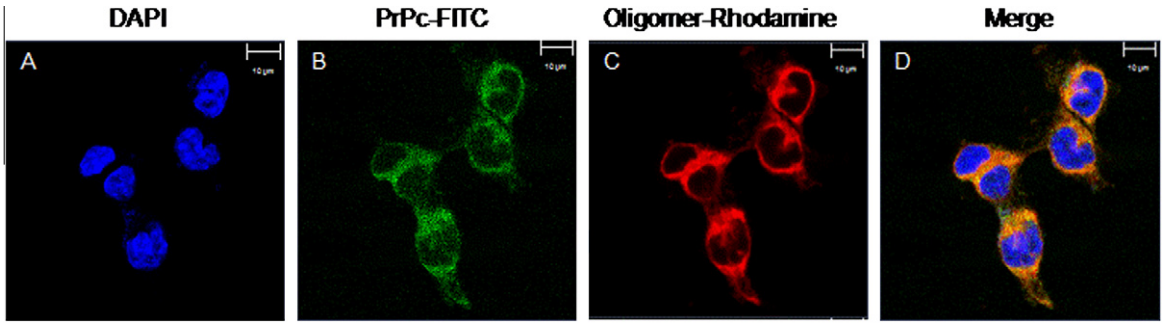

Fig. 4. Immunocytochemical localization of PrPC and $A\beta_{1-42}$ oligomers in L2-2B1 cells were incubated with specific antibodies. (A) The blue color indicates staining of the nuclei. (B) PrPC was distributed throughout the cell, except for nuclei. (C) $A\beta_{1-42}$ oligomers were nearly co-existed to the cell with PrPC. (D) The merged image with three colors. Bar: 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Ca^{2+}-mediated signal transduction [16]. Here, basal $[Ca^{2+}]_I$ concentrations were increased significantly and peaked at around 70% in L2-2B1 cells with addition of 10 μM of $A\beta_{1-42}$ oligomers (Fig. 2C), in contrast to the lowest Ca^{2+} levels observed in HpL 3–4 cells. Therefore, our results suggest that overexpression of PrPC on the cell membrane could synergistically enhance Ca^{2+} release into the cytosol with addition of $A\beta_{1-42}$ oligomers.

In addition to the apoptosis pathway originated from the damaged mitochondria, $A\beta_{1-42}$ oligomers could also mediate the extrinsic apoptosis pathway through caspase-8 activation. Previously, $A\beta_{1-42}$ oligomers have been shown to initiate apoptosis by cross-linking to the death receptors of the Fas/tumor necrosis factor (TNF) family, activating caspase-8 in the extrinsic pathway [17]. Therefore, we investigated the initiation of the extrinsic apoptosis pathway through $A\beta_{1-42}$ oligomer treatment-induced caspase-8 activation in the relative expression of PrPC. In other words, activation of caspase-8 in L2-2B1 and HpL 3–4 cells, respectively, would indicate the involvement of PrPC in the early stages of the extrinsic apoptotic pathway, where $A\beta_{1-42}$ oligomers cross-link to the death receptor and activate procaspase-8 to caspase-8. A study by Lauren et al. demonstrated that $A\beta_{1-42}$ oligomers could bind to the residues 95–110 of PrPC by using anti-PrP antibodies in a competition assay [5]. Another study by Chung et al. suggested that monoclonal PrP antibodies block the binding of $A\beta_{1-42}$ oligomers to PrPC and could be used to treat cognitive deficits in aged AD transgenic mice [18].

Cellular necrosis could be another possible mechanism of the observed neurotoxicity, involving inflammatory responses and associated death pathways in the presence of PrPC and $A\beta_{1-42}$ oligomers. Neuronal disintegration would allow the propagation of death signals to the neighboring cells, triggering an inflammatory response, followed by necrosis. TNF, a cytokine that causes inflammation, is also overlapped in extrinsic apoptosis pathway, where the TNF–TNF receptor 1 complex binds to caspase-8, and it induces eventually apoptosis [19].

The p75 neurotrophin receptor (NTR), a pro-inflammatory cytokine receptor, has also been proposed to be a target of the $A\beta_{1-42}$ oligomers and a prion protein fragment containing the $PrP_{106-126}$ epitope for NF-κB activation [20]. Hence, necrosis by TNF and NF-κB has been implicated in $A\beta_{1-42}$ oligomer-induced cytotoxicity through PrPC. Another study has suggested that necrosis could be initiated from excessive cytosol calcium. The mechanism of PrPC-induced necrosis is not clearly understood, since PrPC alone is not toxic. Our results indicate that overexpressed PrPC could be involved in many processes leading to cytotoxicity. When PrPC-deficient neurons were tested for their responsiveness to staurosporine, a well-known chemical for inducing apoptosis [21]. PrPC-deficient neurons were less responsive. Over- or under-expression of PrPC did not clearly reveal the necessary susceptibility for infection, except where the absence of PrPC correlated well with a lack of transmission. Although many susceptibilities from the genetic polymorphisms of PRNP have been reported [22], the total expression of PrPC should be quantified longitudinally to analyze whether susceptibility and the expression of PrPC are correlated. If toxic agents could interact with PrPC to trigger cytotoxicity, $A\beta_{1-42}$ oligomers or other potential aggregates could interact with PrPC to cause toxicity. In case of AD, a direct correlation was observed between the phosphorylated and total tau proteins in the suspected AD patients. Amplified 14-3-3 protein detection in other neurodegenerative disease, as well as CJD, is also to analyze the amount of 14-3-3 isotypes, which is not toxic [23]. They were marked as disease relative factors despite native protein. Since PrPSc can convert PrPC to PrPSc through structural changes, studies of prion diseases like CJD have focused on the detection of PrPSc for screening and confirming diseases in an effort to eliminate transmission. Investigations of the native function of normal PrPC have been limited; therefore, experiments involving the different PrPC level would be very informative to determine the effect of normal PrPC from cells.

Recently, PrPC has emerged as a receptor for $A\beta_{1-42}$ oligomers [24]. Other neurodegenerative disease markers have been reported for their genetic and physiologic relationships with prion diseases [25]. It has also been suggested that general proteins in cells could non-specifically interact with PrPC, where the interaction between PrPC and the nuclear orphan receptor convert from a protector to a lethal protein through B-cell lymphoma 2 (Bcl-2) [26]. An interesting hypothesis was presented where PrPC, like Bcl-2, could convert from protecting cells to death to being able to initiate apoptosis by a conformational change to PrPSc and by interaction with $A\beta_{1-42}$ oligomers or other potential aggregates [27]. PrPC may not be limited to prion diseases like CJD, but could also play a role as a protector or killer in the pathogenesis of other diseases.

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