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Prion protein impairs kinesin-driven transport

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ABSTRACT

Our previous studies have demonstrated that prion protein (PrP) leads to disassembly of microtubular cytoskeleton through binding to tubulin and its oligomerization. Here we found that PrP-treated cells exhibited improper morphology of mitotic spindles. Formation of aberrant spindles may result not only from altered microtubule dynamics – as expected from PrP-induced tubulin oligomerization – but also from impairing the function of molecular motors. Therefore we checked whether binding of PrP to microtubules affected movement generated by Ncd – a kinesin responsible for the proper organization of division spindles. We found that PrP inhibited Ncd-driven transport of microtubules. Most probably, the inhibition of the microtubule movement resulted from PrP-induced changes in the microtubule structure since Ncd-microtubule binding was reduced already at low PrP to tubulin molar ratios. This study suggests another plausible mechanism of PrP cytotoxicity related to the interaction with tubulin, namely impeding microtubule-dependent transport.

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1. Introduction

The mechanism of a neurotoxic action of prion protein in transmissible spongiform encephalopathies (TSEs) still remains a topic of a hot debate. TSEs are usually manifested by accumulation of misfolded prion protein (PrP^{TSE}) in a form of amyloid plaques, vacuolization, neuronal loss and gliosis in the central nervous system [1]. The neuronal death is preceded by defects in axonal function and synaptic loss [2]. Impairment of axonal transport in cortical motor neurons was observed at the onset of clinical TSE [3]. Noteworthy, defects in the axonal transport have been reported at early stages of numerous other neurodegenerative disorders, such as Alzheimer disease, Huntington disease, Parkinson disease or amyotropic lateral sclerosis [4–7].

Intracellular transport, including axonal, as well as cell divisions are strictly dependent on molecular motors belonging to kinesin and dynein families. These motor proteins employ microtubules as tracks for transport of cargo such as macromolecules, vesicles or organelles. Interestingly, it has been reported that this transport may be affected by non-motile microtubule associated proteins (MAPs), e.g. Tau, known as cytoskeletal stabilizers [8].

A growing body of evidence indicates that PrP, when localized in the cytosol, may interact with tubulin, inhibit microtubule assembly and thereby exert cytotoxic effect [9–11]. In fact, PrP is not only an extracellular protein and its cytosolic mislocalization, increased in some TSEs, may lead to deleterious effects to neurons. These effects are most probably related to interactions of PrP with intracellular proteins [rev. in 12]. It has been reported that PrP co-immunoprecipitates with tubulin in brain extracts [13]. Subsequently, co-localization of the N-terminal fragment of PrP with the microtubular cytoskeleton has been demonstrated in cultured neuroblastoma cells [14]. It has been proposed that this association is related to the active transport of prion protein in neurons, which has been found to be microtubule-dependent [15]. By chemical cross-linking of purified proteins, we have shown that PrP may directly bind to tubulin [9]. Furthermore, analogous to MAPs, PrP co-purifies with tubulin and undergoes co-sedimentation with microtubules. The strong and weak tubulin-binding sites have been mapped within sequence 23–32 and 101–110 of PrP, respectively [11]. This direct interaction leads to oligomerization/aggregation of tubulin and inhibits microtubule assembly *in vitro* [10]. Importantly, a membrane-penetrating PrP peptide 1–30, encompassing the major tubulin binding site, disrupts microtubular cytoskeleton of cultured cells [11]. Disassembly of microtubules by PrP was fur-

Abbreviations: CBB, Coomassie brilliant blue; ^{Ctm}PrP, transmembrane form of prion protein with the C-terminus residing in the lumen of endoplasmic reticulum; ER, endoplasmic reticulum; HRP, horseradish peroxidase; MAPs, microtubule-associated proteins; Ncd, non-claret disjunctional kinesin; PC-12, pheochromocytoma cells; pep1–30, peptide corresponding to PrP sequence 1–30; pep23–32, peptide corresponding to PrP sequence 23–32; pep101–110, peptide corresponding to PrP sequence 101–110; PrP, prion protein; PrP^{TSE}, TSE form of prion protein; TSEs, transmissible spongiform encephalopathies.

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ther confirmed in cells transfected with a plasmid encoding cytosolic form of the protein [16]. Interestingly, it has been also found that pathologic form of prion protein – PrP^{TSE} – co-immunoprecipitates with tubulin in brain homogenates of TSE-infected animals [17]. Moreover, loss of dendritic microtubules has been observed in TSE brain sections [18]. It has also been demonstrated that PrP amyloid fibrils caused collapse of microtubules and tubulin aggregation in neuronal processes [2].

It is plausible that, besides inhibition of microtubule assembly, the direct binding of PrP to tubulin may affect interaction of some MAPs, including molecular motors, with microtubules. One can suppose that microtubule-linked transport may be influenced equally by both mechanisms depending on the cytosolic concentration of PrP and/or PrP conformation. Hence, in this report we examined if PrP, as a microtubule-binding protein, may affect kinesin-driven movement.

2. Materials and methods

2.1. Protein purification

Full-length mature PrP (residues 23–231) was expressed in *Escherichia coli* as a His-tagged polypeptide [19] and purified according to Zahn and colleagues [20]. PrP employed in motility assay was incubated with thrombin-agarose (Sigma, St. Louis, MO, USA) for 2 h at 30 °C to remove His tag, as described previously [11]. After extensive dialysis against deionized water, PrP preparations were cleared by 30 min centrifugation at 200,000*g*, 30 °C. PrP concentration was determined by measuring absorbance at 280 nm using the molar extinction coefficient of 57,870 M^{-1} cm^{-1}.

N-terminally His-tagged dimeric Ncd fragment encompassing residues 250–700 was expressed in *E. coli* and purified as described previously [21].

Porcine brain tubulin was purified by two cycles of polymerization/depolymerization according to the modified method of Mandelkow and colleagues [22], described in details in [9]. The last depolymerization of microtubules was performed for 2 h. Just before experiments, crude tubulin was depleted of endogenous MAPs by incubation in high molarity PIPES buffer as described by Castoldi and Popov [23]. Microtubules were stabilized by 20 μM taxol. Tubulin concentration was measured by Bradford method [24].

Brain Tau was purified from crude tubulin preparations as described previously [25] employing modified methods of Fellous and colleagues [26], and Lindwall and Cole [27]. Concentration of Tau was determined by Bradford method [24].

2.2. Peptide synthesis

PrP peptides: MANLGYWLLALFVTMWTDVGLCKKRPKPGG-NH_2 (pep1–30), KKRPKPGGWN (pep23–32) and KPSKPKTNMK (pep101–110) were synthesized commercially by EZBiolab (Westfield, IN, USA).

2.3. Cell culture and immunocytochemistry

Rat adrenal pheochromocytoma cells (PC-12) purchased from American Type Culture Collection (Manassas, VA, USA) were cultured, treated with 4 μM pep1–30 for 1 h and immunostained with antibodies to β-tubulin (T0198, Sigma) followed by incubation with antibodies to rabbit IgG conjugated with ALEXA 546 (A11003, Invitrogen, Eugene, OR, USA), as described previously [25]. Additionally, DNA was stained with To-Pro3 (T3605, Invitrogen). The cells were observed in confocal microscope Leica TCS SP5.

2.4. Co-sedimentation experiments

Ncd (2 μM) was incubated with taxol-stabilized microtubules (15.6 μM, assuming a molecular weight of 50 kDa for tubulin) in the absence or presence of full-length His-tagged PrP (23–231); in 100 mM NaCl, 1 mM DTT, 1 mM GTP. The mixtures were centrifuged for 30 min at 200,000*g*, 25 °C. Obtained supernatants and pellets were analyzed by Western blotting. Additionally, at the same conditions, effect of the full-length PrP on co-sedimentation of Tau (~2 μM) with microtubules (15.6 μM) was studied, and co-sedimentation was assessed on Coomassie-stained gels.

2.5. Motility assay

Before experiments, tubulin was labeled with tetramethylrhodamine and polymerized into microtubules stabilized by taxol as described previously [21]. Motility assay was described in details by Kocik and colleagues [21]. Ncd was immobilized by anti-His tag antibodies and therefore PrP lacking His tag was used in this assay. The assay was carried out in motility buffer (100 mM potassium acetate, 5 mM magnesium acetate, 0.1 mM EDTA, 0.1 mM EGTA, 5% w/v sucrose, 1 mM ATP, 10 μM taxol, 20 mM Hepes, pH 7.2) supplemented with anti-fade (20 mM D-glucose, 0.02 mg/ml glucose oxidase, 0.008 mg/ml catalase, 100 mM 2-mercaptoethanol) and ~0.2 μM labeled microtubules. In some experiments the motility buffer contained additionally 0.6 μM Tau. The measurements were performed before and after addition of PrP or its peptides, as well as after their removal. To remove PrP/peptides the chamber was washed with the motility buffer (3 volumes of the chamber). The velocity of microtubule movement was measured in ImageJ program and kymographs (space–time plots) were made using MetaMorph software (Molecular Devices, USA).

2.6. Electrophoresis and Western blotting

SDS–PAGE was performed on 10% gels according to the Laemmli method [28]. The blots were analyzed with mouse anti-His tag monoclonal antibodies (05–949, Millipore, Billerica, MA, USA) diluted at 1:1000 followed by goat anti-mouse IgG antibodies (AP308P, Millipore) conjugated with horseradish peroxidase (HRP) diluted at 1:5000. X-ray films (Kodak, Rochester, NY, USA) were exposed to the blots incubated with a chemiluminescent HRP substrate (Millipore). Some gels were stained with Coomassie brilliant blue R (CBB).

3. Results

In previous studies we have demonstrated that PrP interacts directly with tubulin and inhibits microtubule formation by inducement of tubulin oligomerization/aggregation [9–11]. In particular, it has been shown that cell-penetrating peptide [29] corresponding to PrP sequence 1–30 (pep1–30) encompassing signal peptide (residues 1–22) and major tubulin binding site (residues 23–30) disintegrates microtubular cytoskeleton [11,25]. In this report we demonstrated that peptide pep1–30 affected also mitotic spindles in tumor cells of neural origin. As it is shown in Fig. 1, the peptide-treated PC-12 cells form tripolar and multipolar spindles, and in some of the cells spindles are disassembled and tubulin is congregated in a dotted manner. It is reasonable to assume that some of the above aberrations of spindle apparatus result from tubulin oligomerization/aggregation by the PrP peptide. One can however suppose that PrP also competes for tubulin binding with proteins responsible for the proper organization and maintenance of the division spindle e.g. molecular motors.

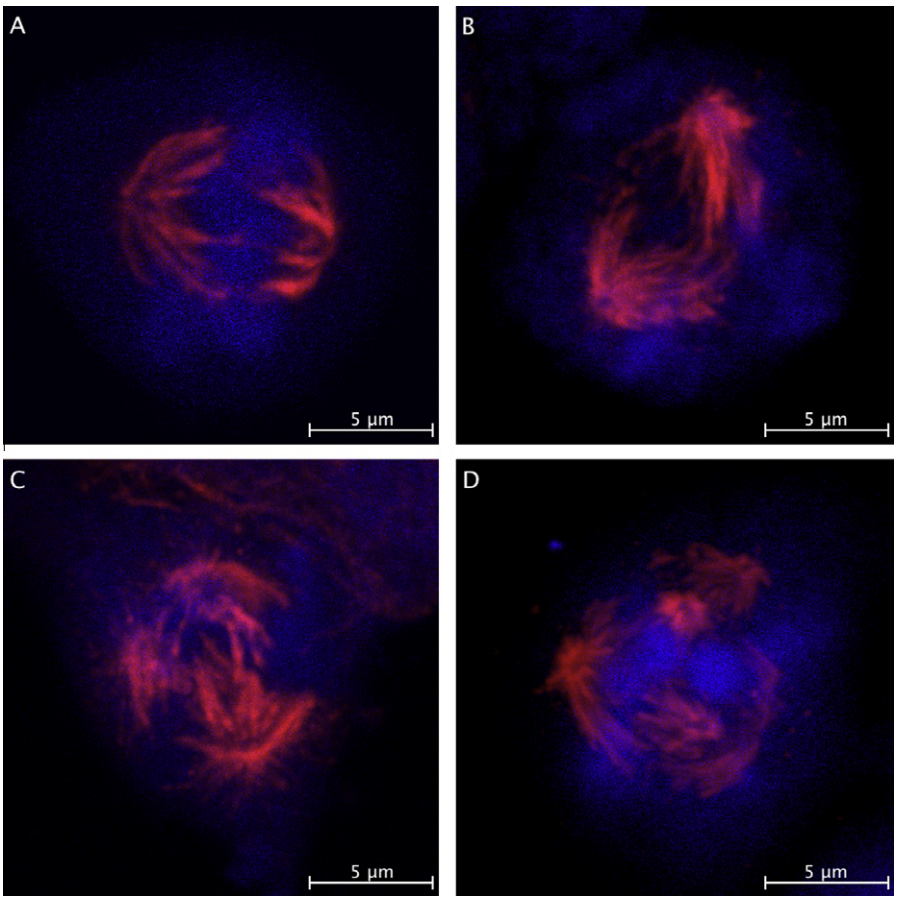

Fig. 1. PrP peptide 1–30 affects division spindles of cultured cells. Treated with 4 μM pep1–30 (B–D) and untreated PC-12 cells (A) were stained with antibodies to β-tubulin to visualize microtubules (red) and To-Pro3 to stain chromosomes (blue). Note multipolar spindles in pep1–30 treated cells (C and D). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

The observation of aberrant mitotic spindles in tumor cells treated with membrane-penetrating PrP peptide has inspired us to check effect of PrP on function of molecular motors associated with microtubules. As a model motor protein we employed Ncd (*non-claret disjunctional*), a kinesin-14 required for assembly of mitotic and meiotic spindles, and chromosome segregation in *Drosophila melanogaster* cells. The effect of PrP on Ncd-driven microtubule transport was assessed by *in vitro* motility assay. To avoid previously reported influence of PrP on microtubule formation all experiments were performed using pre-formed taxol-stabilized microtubules. PrP neither caused aggregation of tubulin nor affected the length of microtubules (data not shown). This indicates that tubulin stabilized by taxol in a form of microtubules is not susceptible to PrP-induced oligomerization. Interestingly, in the motility assay we found that full-length PrP (23–231) significantly reduced the velocity of microtubule gliding by Ncd (Fig. 2, Table 1, and Supplementary movies). Removal of PrP from the assay mixture restored the initial velocity. This points to reversible effect of PrP and excludes aggregation of microtubules.

Subsequently we checked which region of the PrP molecule was responsible for the above described inhibition of Ncd-driven movement. In the motility assay we employed PrP peptides corresponding to the strong (pep23–32) and weak (pep101–110) tubulin binding sites. As shown in Fig. 2 and Table 1, pep23–32 reduced the velocity of microtubule movement whereas pep101–110 had no significant effect. Similarly to the full-length PrP the effect of pep23–32 was reversible and the removal of the peptide restored the initial velocity (Table 1).

Since microtubule associated protein – Tau is able to inhibit PrP-induced oligomerization of tubulin [25] we decided to check whether this protein is also able to influence the above described effect of PrP. Surprisingly, in the motility assay we did not observe any influence of Tau on the effect of PrP on the Ncd-driven movement (Table 2) suggesting that there is no competition between PrP and Tau for binding sites on the taxol-stabilized microtubule. Also Tau alone had no significant effect on the microtubule movement (Table 2).

To gain insight into the mechanism of PrP action we checked whether this protein influenced binding of Ncd to microtubules and thereby might affect cell division. We performed co-sedimentation experiments where taxol-stabilized pre-formed microtubules were incubated with Ncd in the presence of increasing concentrations of full-length PrP. Since tubulin and Ncd co-migrate during electrophoresis, obtained supernatants and pellets were analyzed on Western blots using anti-His tag antibodies to visualize tagged Ncd. As demonstrated in Fig. 3A, PrP reduced the amount of Ncd co-sedimented with microtubules. Notably, this effect was observed already at 1:39 PrP to tubulin molar ratio. At the same time, PrP had neither effect on sedimentation of microtubules nor solubility of Ncd alone (data not shown). As expected from the motility experiments, at the same PrP concentrations (and even higher), co-sedimentation of Tau with microtubules was unaffected (Fig. 3B). This again suggests that there is no competition between PrP and Tau for binding sites on microtubules, whereas PrP specifically affects interaction of the motor protein with microtubules.

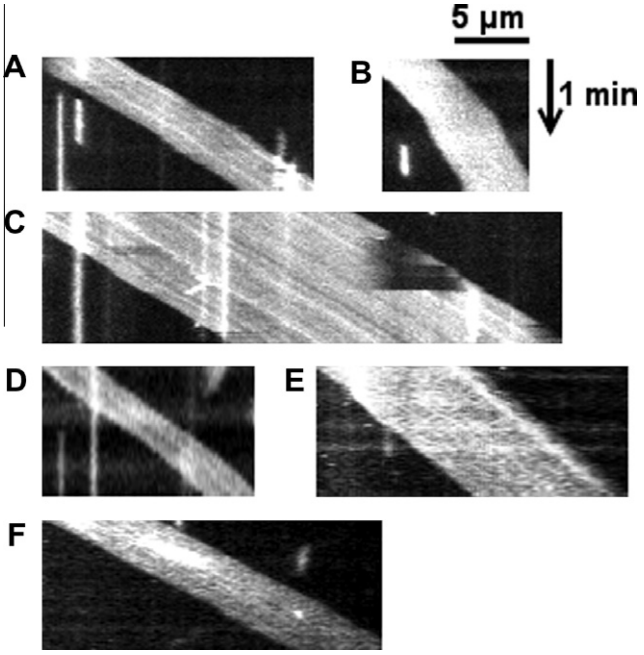

Fig. 2. PrP inhibits Ncd-driven movement of microtubules in motility assay. Rhodamine-labeled microtubules (~0.2 μM) moving over a Ncd-coated surface were observed under fluorescence microscope. Kymographs for motility assay in the absence of PrP (A), in the presence of 4.5 μM full-length PrP (B), 50 μM PrP peptide 23–32 (D), 660 μM PrP peptide 23–32 (E) and 660 μM PrP peptide 101–110 (F) are shown. Additionally, a kymograph obtained after removal of full-length PrP is presented (C). Each kymograph describes the behavior of a selected microtubule during *in vitro* motility assay. The slope of the kymograph track relative to the time axis represents the velocity of the microtubule.

Table 1
PrP inhibits Ncd-driven movement of microtubules in motility assay.

	Velocity [nm/s][a]	Velocity after removal of PrP/peptides [nm/s] [a]
Ncd	160 ± 26	–
Ncd + 4.5 μM full-length PrP	57 ± 17	160 ± 30
Ncd + 50 μM pep23–32	134 ± 14	177 ± 11
Ncd + 660 μM pep23–32	97 ± 17	185 ± 16
Ncd + 50 μM pep101–110	166 ± 11	167 ± 17
Ncd + 660 μM pep101–110	144 ± 16	168 ± 33

[a] Mean velocities ± standard deviation were calculated for all microtubules in a field of view.

Table 2
Tau has no effect on PrP-caused inhibition of Ncd-driven movement of microtubules in motility assay.

	Velocity [nm/s][a]	Velocity after removal of PrP/Tau [nm/s] [a]
Ncd	146 ± 17	–
Ncd + 2 μM full-length PrP	108 ± 24	143 ± 32
Ncd + 0.6 μM Tau	121 ± 16	138 ± 18
Ncd + 2 μM full-length PrP + 0.6 μM Tau	105 ± 17	126 ± 12

[a] Mean velocities ± standard deviation were calculated for all microtubules in a field of view.

4. Discussion

In this study we have demonstrated that PrP affects Ncd-driven microtubule gliding. What is the mechanism of the movement inhibition? In co-sedimentation experiments we have shown that PrP reduces the binding of Ncd to microtubules. This effect is observed already at low PrP to tubulin molar ratios – leaving

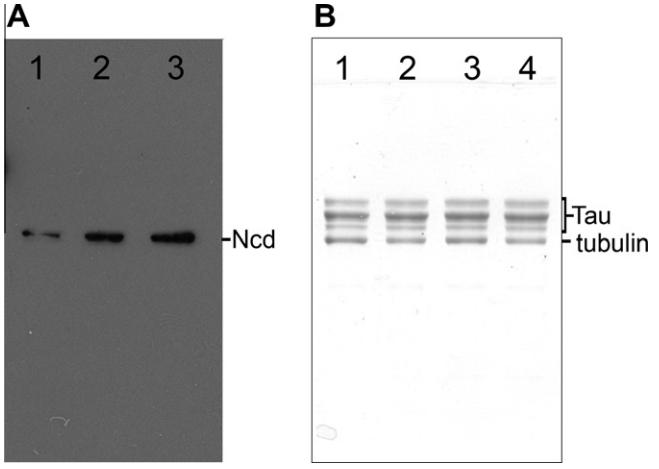

Fig. 3. PrP reduces co-sedimentation of Ncd with microtubules. In (A) Ncd (2 μM) was centrifuged with microtubules (15.6 μM) in the absence (1) and in the presence of 0.4 μM (2) and 0.6 μM (3) full-length PrP. Western blot analyzed with anti-His tag antibody shows tagged Ncd remained in the supernatants. In (B) it is demonstrated that PrP has no effect on co-sedimentation of Tau (~2 μM) with microtubules (15.6 μM). CBB-stained gel shows Tau in the supernatants obtained after centrifugation in the absence (1) and in the presence of 0.4 μM (2), 0.6 μM (3) and 0.8 μM (4) full-length PrP.

numerous microtubule subunits free to interact with Ncd. It is therefore rather unlikely that the mechanism is solely based on the competition between PrP and Ncd for binding sites on the microtubule. Most plausibly, PrP exerts its effect by inducing structural changes in the microtubule. This is in accordance with previously reported oligomerization of tubulin by PrP which inhibits microtubule formation [10]. Herein, to avoid oligomerization of tubulin, we used taxol-stabilized microtubules. However, it is reasonable to expect that some structural changes in the microtubule may still occur upon binding of PrP. Interestingly, as small as 10-aa PrP peptide, corresponding to major tubulin binding site (PrP residues 23–32), is able to hinder Ncd-driven transport. This peptide has also been shown to induce oligomerization of tubulin [11].

As we demonstrated recently [25], Tau reduced oligomerization of tubulin induced by PrP apparently through stabilization of structures formed by tubulin since we did not observe competition between Tau and PrP for binding site on unpolymerized tubulin. Herein we show additionally that, at the conditions revealing the reduction in Ncd-microtubule binding, there is no effect of PrP on the binding of Tau to the microtubule. Our results may suggest that the binding sites for Tau and Ncd do not fully overlap since only binding of Ncd is affected by PrP. Noteworthy, Tau has been shown in some reports to reduce kinesin-1 mediated transport. However, the mechanism of this effect remains unclear [8]. Recent studies have demonstrated that Tau may function as an inhibitory or non-inhibitory factor depending on its isoform and structural state of the microtubule [30].

Intracellular localization of PrP is thought to be neurotoxic [31–33]. In healthy organisms PrP is a glycoprotein residing mostly on the cell surface [34]. Exceptions are some subpopulations of neurons in hippocampus, neocortex and thalamus where PrP has been found predominantly in the cytosol [35]. In general, elevated intracellular concentration of PrP is related to pathology. Mutations within hydrophobic domain of PrP, linked to some familial forms of TSE, increase the generation of a transmembrane form of PrP (^{Ctm}PrP) associated with endoplasmic reticulum (ER). The N-terminal domain of ^{Ctm}PrP is exposed to the cytosol whereas the C-terminal domain is localized in the ER lumen [31,36,37, rev. in 38]. Cytosolic accumulation of PrP may also result from stop mutations leading to synthesis of C-terminally truncated molecules [39,40], affected translocation of nascent PrP [41], inhibition of the protea-

some by PrP^{TSE} [41,42] and the ER stress [rev. in 43], all observed in TSEs. It is postulated that exposition of intracellular proteins to mislocalized PrP leading to loss or modification of their physiological functions underlies mechanism of the neurotoxic effect [rev. in 43 and 12]. Accordingly, it has been reported that PrP induces aggregation of several cytosolic proteins of crucial cellular function, e.g. Bcl-2, mahogunin and tubulin [44,45,10,11].

Since PrP is not exclusively a neural molecule and prion-linked pathogenesis is not restricted only to the nervous system it is plausible that PrP may influence function of mitotic spindles in non-neural cells. In fact, PrP has been shown to negatively regulate mitosis in epithelial cells. It has been demonstrated that human enterocytes treated with PrP-si-RNA exhibit higher mitotic frequency [46].

Furthermore, it is very plausible that PrP, as a microtubule-binding protein, affects not only function of molecular motors involved in cell divisions but also kinesins responsible for intracellular transport. Therefore, we further hypothesize that disordered axonal transport, observed in TSEs, may result from PrP-related impairment of kinesin function, particularly in those diseases where cytosolic PrP is generated. Interestingly, defects in axonal transport have been widely reported in Alzheimer disease [6] as well as PrP-related pathology in some familial forms of this disease [47].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.153.

References

[1] S.B. Prusiner, Prions, Proc. Natl. Acad. Sci. USA 95 (1998) 13363–13383.
[2] V. Novitskaya, N. Makarava, I. Sylvester, I.B. Bronstein, I.V. Baskakov, Amyloid fibrils of mammalian prion protein induce axonal degeneration in NTERA2-derived terminally differentiated neurons, J. Neurochem. 102 (2007) 398–407.
[3] V. Ermolayev, M. Friedrich, R. Nozadze, T. Cathomen, M.A. Klein, G.S. Harms, E. Flechsig, Ultramicroscopy reveals axonal transport impairments in cortical motor neurons at prion disease, Biophys. J. 96 (2009) 3390–3398.
[4] L.S. Goldstein, Do disorders of movement cause movement disorders and dementia?, Neuron 40 (2003) 415–425
[5] S. Gunawardena, L.S. Goldstein, Polyglutamine diseases and transport problems: deadly traffic jams on neuronal highways, Arch. Neurol. 62 (2005) 46–51.
[6] G.B. Stokin, L.S. Goldstein, Axonal transport and Alzheimer's disease, Annu. Rev. Biochem. 75 (2006) 607–627.
[7] S. Roy, B. Zhang, V.M. Lee, J.Q. Trojanowski, Axonal transport defects: a common theme in neurodegenerative diseases, Acta Neuropathol. 109 (2005) 5–13.
[8] R. Dixit, J.L. Ross, Y.E. Goldman, E.L. Holzbaur, Differential regulation of dynein and kinesin motor proteins by tau, Science 319 (2008) 1086–1089.
[9] K. Nieznanski, H. Nieznanska, K.J. Skowronek, K.M. Osiecka, D. Stepkowski, Direct interaction between prion protein and tubulin, Biochem. Biophys. Res. Commun. 334 (2005) 403–411.
[10] K. Nieznanski, Z.A. Podlubnaya, H. Nieznanska, Prion protein inhibits microtubule assembly by inducing tubulin oligomerization, Biochem. Biophys. Res. Commun. 349 (2006) 391–399.
[11] K.M. Osiecka, H. Nieznanska, K.J. Skowronek, J. Karolczak, G. Schneider, K. Nieznanski, Prion protein region 23–32 interacts with tubulin and inhibits microtubule assembly, Proteins 77 (2009) 279–296.
[12] K. Nieznanski, Interactions of prion protein with intracellular proteins: so many partners and no consequences?, Cell Mol. Neurobiol. 30 (2010) 653–666.
[13] G.I. Keshet, O. Bar-Peled, D. Yaffe, U. Nudel, R. Gabizon, The cellular prion protein colocalizes with the dystroglycan complex in the brain, J. Neurochem. 75 (2000) 1889–1897.
[14] N.S. Hachiya, K. Watanabe, Y. Sakasegawa, K. Kaneko, Microtubules-associated intracellular localization of the NH_2-terminal cellular prion protein fragment, Biochem. Biophys. Res. Commun. 313 (2004) 818–823.
[15] N.S. Hachiya, K. Watanabe, M. Yamada, Y. Sakasegawa, K. Kaneko, Anterograde and retrograde intracellular trafficking of fluorescent cellular prion protein, Biochem. Biophys. Res. Commun. 315 (2004) 802–807.
[16] X.L. Li, G.R. Wang, Y.Y. Jing, M.M. Pan, C.F. Dong, R.M. Zhou, Z.Y. Wang, Q. Shi, C. Gao, X.P. Dong, Cytosolic PrP induces apoptosis of cell by disrupting microtubule assembly, J. Mol. Neurosci. 43 (2011) 316–325.
[17] C.F. Dong, S. Shi, X.F. Wang, R. An, P. Li, J.M. Chen, X. Wang, G.R. Wang, B. Shan, B.Y. Zhang, J. Han, X.P. Dong, The N-terminus of PrP is responsible for interacting with tubulin and fCJD related PrP mutants possess stronger inhibitive effect on microtubule assembly in vitro, Arch. Biochem. Biophys. 470 (2008) 83–92.
[18] E.G. Gray, Spongiform encephalopathy: a neurocytologist's viewpoint with a note on Alzheimer's disease, Neuropathol. Appl. Neurobiol. 12 (1986) 149–172.
[19] M. Morillas, W. Swietnicki, P. Gambetti, W.K. Surewicz, Membrane environment alters the conformational structure of the recombinant human prion protein, J. Biol. Chem. 274 (1999) 36859–36865.
[20] R. Zahn, C. von Schroetter, K. Wuthrich, Human prion proteins expressed in *Escherichia coli* and purified by high-affinity column refolding, FEBS Lett. 417 (1997) 400–404.
[21] E. Kocik, K.J. Skowronek, A.A. Kasprzak, Interactions between subunits in heterodimeric Ncd molecules, J. Biol. Chem. 284 (2009) 35735–35745.
[22] E.M. Mandelkow, M. Herrmann, U. Ruhl, Tubulin domains probed by limited proteolysis and subunit-specific antibodies, J. Mol. Biol. 185 (1985) 311–327.
[23] M. Castoldi, A.V. Popov, Purification of brain tubulin through two cycles of polymerization–depolymerization in a high-molarity buffer, Protein Expr. Purif. 32 (2003) 83–88.
[24] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[25] K.M. Osiecka, H. Nieznanska, K.J. Skowronek, J. Jozwiak, K. Nieznanski, Tau inhibits tubulin oligomerization induced by prion protein, Biochim. Biophys. Acta 2011 (1813) 1845–1853.
[26] A. Fellous, J. Francon, A.M. Lennon, J. Nunez, Microtubule assembly in vitro. Purification of assembly-promoting factors, Eur. J. Biochem. 78 (1977) 167–174.
[27] G. Lindwall, R.D. Cole, The purification of tau protein and the occurrence of two phosphorylation states of tau in brain, J. Biol. Chem. 259 (1984) 12241–12245.
[28] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[29] P. Lundberg, M. Magzoub, M. Lindberg, M. Hällbrink, J. Jarvet, L.E. Eriksson, U. Langel, A. Gräslund, Cell membrane translocation of the N-terminal (1–28) part of the prion protein, Biochem. Biophys. Res. Commun. 299 (2002) 85–90.
[30] D.P. McVicker, L.R. Chrin, C.L. Berger, The nucleotide-binding state of microtubules modulates kinesin processivity and the ability of Tau to inhibit kinesin-mediated transport, J. Biol. Chem. 286 (2011) 42873–42880.
[31] R.S. Hegde, J.A. Mastrianni, M.R. Scott, K.A. DeFea, P. Tremblay, M. Torchia, S.J. DeArmond, S.B. Prusiner, V.R. Lingappa, A transmembrane form of the prion protein in neurodegenerative disease, Science 279 (1998) 827–834.
[32] J. Ma, R. Wollmann, S. Lindquist, Neurotoxicity and neurodegeneration when PrP accumulates in the cytosol, Science 298 (2002) 1781–1785.
[33] C. Grenier, C. Bissonnette, L. Volkov, X. Roucou, Molecular morphology and toxicity of cytoplasmic prion protein aggregates in neuronal and non-neuronal cells, J. Neurochem. 97 (2006) 1456–1466.
[34] N. Stahl, D.R. Borchelt, S.B. Prusiner, Differential release of cellular and scrapie prion proteins from cellular membranes by phosphatidylinositol-specific phospholipase C, Biochemistry 29 (1990) 5405–5412.
[35] A. Mironov Jr., D. Latawiec, H. Wille, E. Bouzamondo-Bernstein, G. Legname, R.A. Williamson, D. Burton, S.J. DeArmond, S.B. Prusiner, P.J. Peters, Cytosolic prion protein in neurons, J. Neurosci. 23 (2003) 7183–7193.
[36] C.S. Yost, C.D. Lopez, S.B. Prusiner, R.M. Myers, V.R. Lingappa, Non-hydrophobic extracytoplasmic determinant of stop transfer in the prion protein, Nature 343 (1990) 669–672.
[37] S.J. Kim, R. Rahbar, R.S. Hegde, Combinatorial control of prion protein biogenesis by the signal sequence and transmembrane domain, J. Biol. Chem. 276 (2001) 26132–26140.
[38] O. Chakrabarti, A. Ashok, R.S. Hegde, Prion protein biosynthesis and its emerging role in neurodegeneration, Trends Biochem. Sci. 34 (2009) 287–295.
[39] G. Zanusso, R.B. Petersen, T. Jin, Y. Jing, R. Kanoush, S. Ferrari, P. Gambetti, N. Singh, Proteasomal degradation and N-terminal protease resistance of the codon 145 mutant prion protein, J. Biol. Chem. 274 (1999) 23396–23404.
[40] J. Heske, U. Heller, K.F. Winklhofer, J. Tatzelt, The C-terminal globular domain of the prion protein is necessary and sufficient for import into the endoplasmic reticulum, J. Biol. Chem. 279 (2004) 5435–5443.

[41] J. Ma, S. Lindquist, Wild-type PrP and a mutant associated with prion disease are subject to retrograde transport and proteasome degradation, Proc. Natl. Acad. Sci. USA 98 (2001) 14955–14960.
[42] M. Kristiansen, P. Deriziotis, D.E. Dimcheff, G.S. Jackson, H. Ovaa, H. Naumann, A.R. Clarke, F.W. van Leeuwen, V. Menéndez-Benito, N.P. Dantuma, J.L. Portis, J. Collinge, S.J. Tabrizi, Disease-associated prion protein oligomers inhibit the 26S proteasome, Mol. Cell 26 (2007) 175–188.
[43] M. Miesbauer, A.S. Rambold, K.F. Winklhofer, J. Tatzelt, Targeting of the prion protein to the cytosol: mechanisms and consequences, Curr. Issues Mol. Biol. 12 (2009) 109–118.
[44] A.S. Rambold, M. Miesbauer, D. Rapaport, T. Bartke, M. Baier, K.F. Winklhofer, J. Tatzelt, Association of Bcl-2 with misfolded prion protein is linked to the toxic potential of cytosolic PrP, Mol. Biol. Cell 17 (2006) 3356–3368.
[45] O. Chakrabarti, R.S. Hegde, Functional depletion of mahogunin by cytosolically exposed prion protein contributes to neurodegeneration, Cell 137 (2009) 1136–1147.
[46] E. Morel, S. Fouquet, C. Strup-Perrot, C.P. Thievend, C. Petit, D. Loew, A.M. Faussat, L. Yvernault, M. Pincon-Raymond, J. Chambaz, M. Rousset, S. Thenet, C. Clair, The cellular prion protein PrP is involved in the proliferation of epithelial cells and in the distribution of junction-associated proteins, PLoS ONE 3 (2008) e3000.
[47] G. Leuba, K. Saini, A. Savioz, Y. Charnay, Early-onset familial Alzheimer disease with coexisting beta-amyloid and prion pathology, JAMA 283 (2000) 1689–1691.