Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 374 (2005) 98–103
Neuroscience Letters
www.elsevier.com/locate/neulet

Mitochondrial localization of cellular prion protein (PrP^C) invokes neuronal apoptosis in aged transgenic mice overexpressing PrP^C

Naomi S. Hachiya[a,b], Makiko Yamada[a,b], Kota Watanabe[a,b], Akiko Jozuka[a,b], Takuya Ohkubo[a,c], Kenichi Sano[a,1], Yoshio Takeuchi[a,2], Yoshimichi Kozuka[d], Yuji Sakasegawa[a], Kiyotoshi Kaneko[a,b,*]

[a] *Departments of a Cortical Function Disorders, National Institute of Neuroscience (NIN), National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo 187-8502, Japan*
[b] *Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan*
[c] *Department of Neurology and Neurological Science, Graduate School of Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-0034, Japan*
[d] *Ultrastructural Research, National Institute of Neuroscience (NIN), National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo 187-8502, Japan*

Received 14 September 2004; received in revised form 12 October 2004; accepted 13 October 2004

Abstract

Recent studies suggest that the disease isoform of prion protein (PrP^{Sc}) is non-neurotoxic in the absence of cellular isoform of prion protein (PrP^C), indicating that PrP^C may participate directly in the neurodegenerative damage by itself. Meanwhile, transgenic mice harboring a high-copy-number of wild-type mouse (Mo) PrP^C develop a spontaneous neurological dysfunction in an age-dependent manner, even without inoculation of PrP^{Sc} and thus, investigations of these aged transgenic mice may lead to the understanding how PrP^C participate in the neurotoxic property of PrP. Here we demonstrate mitochondria-mediated neuronal apoptosis in aged transgenic mice overexpressing wild-type $MoPrP^C$ (Tg(MoPrP)4053/FVB). The aged mice exhibited an aberrant mitochondrial localization of PrP^C concomitant with decreased proteasomal activity, while younger littermates did not. Such aberrant mitochondrial localization was accompanied by decreased mitochondrial manganese superoxide dismutase (Mn-SOD) activity, cytochrome *c* release into the cytosol, caspase-3 activation, and DNA fragmentation, most predominantly in hippocampal neuronal cells. Following cell culture studies confirmed that decrease in the proteasomal activity is fundamental for the PrP^C-related, mitochondria-mediated apoptosis. Hence, the neurotoxic property of PrP^C could be explained by the mitochondria-mediated neuronal apoptosis, at least in part.

Keywords: PrP^C; Proteasomal activity; Mitochondrial localization; Superoxide dismutase activity; Mitochondria-mediated apoptosis

The posttranslational conformational change of the cellular isoform of prion protein (PrP^C) into its scrapie isoform (PrP^{Sc}) is the fundamental process underlying the pathogenesis of prion diseases [24], but the molecular events through which prion infection and the resulting accumulation of PrP lead to the neuronal dysfunction, vacuolation, and death that characterize prion pathology remain unclear [6].

Importantly, PrP^{Sc}, the disease isoform of PrP, seems to be non-neurotoxic in the absence of PrP^C, suggesting that PrP^C may participate directly in the prion neurodegenerative damage by itself, and the cellular pathways activated by neurotoxic forms of PrP that ultimately result in neuronal death are also being investigated, and several possible mechanisms have been uncovered [6]. For example, cross-linking

* Corresponding author. Tel.: +81 42 346 1718; fax: +81 42 346 1748.
E-mail address: kaneko@ncnp.go.jp (K. Kaneko).
[1] Present address: Hinoieda Kagaku Ltd., Hino-city, Tokyo 191-0061, Japan.
[2] Present address: KOHJIN-BIO Ltd., Sakado-city, Saitama 350-0214, Japan.

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.10.044

PrP^C in vivo with specific monoclonal antibodies was found to trigger neuronal apoptosis, suggesting that PrP^C functions in the control of neuronal survival [26]. In fact, neural tissues overexpressing PrP^C grafted into the brains of PrP^C-deficient mice develop the severe histopathological changes characteristic of prion disease when infected with prions, but no pathological changes were seen in PrP^C-deficient tissue, not even in the immediate vicinity of the grafts despite the presence of high levels of PrP^{Sc} [2]. In addition, interruption of PrP^C expression during an ongoing prion infection prevents neuronal loss and reverses early spongiform change [16]. The continued accumulation of PrP^{Sc} in this model after neuronal PrP^C depletion is likely to reflect prion replication predominantly in both microglia and astrocytes glial cells without PrP^C depletion, which support PrP^{Sc} replication. The PrP^{Sc} deposits colocalize with astrocytes in the brains of infected mice with neuronal PrP^C depletion, which was not seen in scrapie-infected control animals without PrP depletion. The fact that these mice remain asymptomatic indicates that even extensive extraneuronal PrP^{Sc} replication does not cause clinical disease or neurodegeneration in this model. Thus, neuronal PrP^C seems to be fundamental for the neurotoxic property of PrP even in the PrP^{Sc}-infected conditions, but the detailed molecular events especially with non-mutant, wild-type PrP^C still remained unclear.

Meanwhile, aged transgenic mice harboring a high-copy-number of wild-type PrP-B transgenes spontaneously developed mitochondrial encephalomyopathy including focal vacuolation of the central nervous system, skeletal muscles and peripheral nerves without PrP^{Sc} inoculation [28]. Such focal vacuolation was localized to the hippocampus, the superior colliculus, and midbrain tegmentum, which resembled that seen in experimental scrapie, albeit less intense. Other transgenic lines harboring a high-copy-number of wild-type PrP transgenes also exhibited spontaneous neurological dysfunction in an age-dependent manner [21,27]. For example, transgenic mice overexpressing the wild-type mouse (Mo) PrP-A gene (Tg(MoPrP)4053/FVB) used in this study became symptomatic at around the age of 700 days, although no pathological evidence for prion diseases was evident [27]. Since no PrP^{Sc} has been inoculated in these mice, investigations of these aged transgenic mice overexpressing wild-type PrP^C may lead to the better understanding how PrP^C participate in the neurotoxic property of PrP.

Here we show that the Tg(MoPrP)4053/FVB mice exhibited an aberrant mitochondrial localization of PrP^C accompanied by decreased mitochondrial manganese superoxide dismutase (Mn-SOD) activity, cytochrome *c* release in the cytosol, caspase-3 activation, and DNA fragmentation, concomitant with decreased proteasomal activity in an age-dependent manner.

Tg(MoPrP)4053/FVB and its littermate were kindly provided by Dr. S.B. Prusiner (University of California, San Francisco). Antibodies K3 and K4 against PrP were rabbit polyclonal sera raised against PrP peptides corresponding to residues 76–90 and 96–110 in MoPrP, respectively. Anti-cytochrome *c* and anti-porin antibodies were purchased from BD Biosciences. Anti-Hsc70 antibody was purchased from Stressgen Biotechnologies Corporation. Mitotracker Red CMXRos was purchased from Molecular Probes. Lactacystin, ALLN, and MG132 were purchased from Sigma. The $\Delta\Psi m$ detection kit and APO-BrdU TUNEL assay kit were purchased from Trevigen Inc. and Molecular Probes, respectively. Antibodies were used at 1:1000 (Western blotting) or 1:100 (immunofluorescence microscopy) unless otherwise noted. For immuno-electronmicroscopy, 10 nm golds were purchased from DAKO.

Cells or brains were homogenized with 9 volumes of mitochondrial buffer (220 mM mannitol, 70 mM sucrose, 10 mM Hepes-KOH, pH 7.4, and 0.1 mM EDTA) and centrifuged at $700 \times g$ for 5 min at 4 °C, and the supernatant was further centrifuged at $5000 \times g$ for 10 min at 4 °C. The supernatant was used as a post-mitochondrial supernatant. The resulted pellet was washed three times with mitochondrial buffer, resuspended in 9 volumes of the same buffer, and then centrifuged at $2000 \times g$ for 2 min at 4 °C followed by $5000 \times g$ for 8 min at 4 °C. The pellet was resuspended in 9 volumes of the same buffer, and then centrifuged at $5000 \times g$ for 10 min at 4 °C. The final pellet was recovered and stored on ice until use (mitochondrial fraction). The post-mitochondrial supernatant was further centrifuged at $100{,}000 \times g$ for 1 h at 4 °C, and the supernatant was used as cytosolic fraction, and the pellet was resuspended in mitochondrial buffer (microsome fraction). Western blots were performed at 5 μg of total protein/lane.

Mitochondrial manganese superoxide dismutase (Mn-SOD) and cytosolic copper/zinc SOD (Cu/Zinc-SOD) activities were measured by the SOD assay kit (Dojindo Molecular Technologies, Inc.), and cytosolic glutathione (GSH) was measured by the Glutathione quantification kit (Dojindo Molecular Technologies, Inc.) according to the manufacturer's instructions. Caspase-3 activity was measured using the PARP Western Blot Kit (WAKO) according to the manufacturer's instructions. DNA fragmentation was measured by the TUNEL assay (ApopTag® Peroxidase *In situ* Apoptosis Detection Kit, CHEMICON International), which was performed according to the manufacturer's instructions before being visualized with an Olympus CX40 (Olympus Optical Co., Ltd.). Sections were counter-stained by 0.5% methyl green (WAKO) in 0.1 M sodium acetate (pH 4.0).

Proteasomal activity assay was performed as previously described [3,9,31].

Tg(MoPrP)4053/FVB harboring a high-copy-number of wild-type PrP-A transgenes at the age of 520 days (TG520) and an age-matched non-transgenic littermate (WT520) showed similar migration rates of PrP^C on poly acrylamide gel electrophoresis and Western blotting using anti-PrP-antibody K4 (Fig. 1A, PK(−)). As increased resistance to protease K digestion is often a feature of PrP^{Sc}, this was examined in TG520 and WT520. No resistance to proteinase K digestion was detected in any of these mice (Fig. 1A, PK(+)). Histological examinations of the TG520 brains including