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# Down-regulation of brain-derived neurotrophic factor and its signaling components in the brain tissues of scrapie experimental animals

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ABSTRACT

Prion is a unique nucleic acid-free pathogen that causes human and animal fatal neurodegenerative diseases. Brain-derived neurotrophic factor (BDNF) is a prototypic neurotrophin that helps to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses through axonal and dendritic sprouting. There are two distinct classes of glycosylated receptors, neurotrophin receptor p75 (p75NTR) and tropomyosin-related kinase (Trk), that can bind to BDNF. To obtain insights into the possible alterations of brain BDNF and its signaling pathway in prion disease, the levels of BDNF and several molecules in the BDNF pathway in the brain tissues of scrapie agents 263K-infected hamsters were separately evaluated. Western blots and/or immunohistochemical (IHC) assays revealed that BDNF, TrkB, GRB2 and p75NTR, were significantly downregulated in the brain tissues of scrapie-infected rodents at terminal stage. Double-stained immunofluorescent assay (IFA) demonstrated that BDNF and phospho-TrkB predominately expressed in neurons. Dynamic analyses of the brain samples collected at the different time-points during the incubation period illustrated continuous decreases of BDNF, TrkB, phospho-TrkB, GRB2 and p75NTR, which correlated well with neuron loss. However, these proteins remained almost unchanged in the prion infected cell line SMB-S15 compared with those of its normal cell line SMB-PS. These data suggest that the BDNF signaling pathway is severely hindered in the brains of prion disease, which may contribute, at least partially, to the neuron death.



## 1. Introduction

Prion diseases are a group of fatal and transmissible neurodegenerative diseases which affect a broad range of mammals. Human prion diseases are highly heterogeneous, including sporadic, genetic and infectiously acquired forms. Animal prion diseases mainly include scrapie in sheep and goat, chronic wasting disease in deer, elk and moose, and bovine spongiform encephalopathy in cattle. They are characterized by progressive neuronal degeneration, neuronal vacuolation and gliosis. The conversion of normal cellular prion ($PrP^{C}$) to abnormal form of scrapie prion ($PrP^{Sc}$) is believed to be the critical event in prion pathogenesis (Fischer et al., 1992; Jc et al., 1994; Legname et al., 2004).

Neurotrophins are small proteins vital for neuronal growth, differentiation, survival and plasticity (Schindowski et al., 2008). Nerve growth factor (NGF), brain-derived neurotrophin factor (BDNF) and neurotrophin-3 (NT-3) are members of the neurotrophin protein family and act through their cognate

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tropomyosin-related kinase (Trk) receptors (NGF/TrkA, BDNF/TrkB, NT-3/TrkC) and the common neurotrophin receptor p75 (p75NTR) (Gao C, Huang and Reichardt, 2001, Kaplan and Miller, 2000, Lewin and Barde, 1996). In particular, BDNF and sometimes NT-3, appear rapid, primarily presynaptic effects at central, autonomic, and neuromuscular synapses and produce long-term presynaptic and postsynaptic changes consistent with altered gene expression (Poo, 2001; Schuman, 1999).

TrkB regulates growth and survival of the cells by controlling the Ras-PI3K-Akt signaling cascade. In neurons, TrkB may act on an adaptor protein GRB2 and activate the GRB2-Ras-MAPK-Erk signaling in order to regulate the neuronal differentiation (Cowley et al., 1994; Meakin et al., 1999). The pan-neurotrophin receptor, p75NTR promotes distinct signaling pathways in the cells that in most cases oppose but sometimes coordinate with TrkB promoted pathways. p75NTR modulates TrkB actions through influencing the receptor conformation and subsequently altering TrkB specificity and affinity with neurotrophins under physiological and pathological conditions (Huang and Reichardt, 2003).

Loss of TrkB signaling is reported to play roles in pathogenesis of Alzheimer's, Huntington's and other neurodegenerative disorders. It has been reported that in the brains of Alzheimer's disease (AD) animal models and patients, the levels of BDNF are decreased in the entorhinal cortex and hippocampus (Connor et al., 1996). In addition, studies have pointed out the correlations between alteration of BDNF and mechanisms of a serials of disorders, such as Huntington disease (Zuccato and Cattaneo, 2007), Parkinson disease (Fumagalli et al., 2006), Rett syndrome (Ogier et al., 2007), traumatic brain injury (TBI) (Griesbach et al., 2009a,b), and aging (Magnus and Mattson, 2006). Despite the important roles of BDNF and TrkB in multiply biological and pathological processes, the situations of brain BDNF/TrkB during prion infection remain unclear.

Our previous studies of global gene expressions with microarray have revealed down-regulated transcriptional levels of BDNF and TrkB in the brain tissues of some human genetic prion diseases (Tian et al., 2013). In the current study, the protein levels of BDNF and TrkB in the brain tissues of scrapie agent 263K-infected hamsters were evaluated with various methodologies. We found that the brain levels of BDNF, as well as TrkB, p-TrkB, GRB2 and p75NTR in the 263K-infected hamsters were significantly decreased at the terminal stage of disease. Confocal microscopy showed that BDNF and p-TrkB signals co-localized exactly with the NeuN-stained cells, indicating that the cells expressing BDNF and p-TrkB are neurons. Dynamic analysis of the 263K-infected samples collected at different time-points during incubation period illustrated a time-dependent reduction of BDNF and the relevant factors.

## 2. Materials and methods

### 2.1. Ethics statement

Usage of the storage animal brain specimens in this study was approved by the Ethical Committee of National Institute for Viral Disease Prevention and Control, China CDC.

### 2.2. Reagents and antibodies

The following antibodies were used in this study, including anti-β-actin mouse monoclonal antibody (mAb) (Santa Cruz, USA), rabbit anti-BDNF polyclonal antibody (pAb) (Santa Cruz, sc-546), rabbit-anti-TrkB pAb (80E3, #4603S), rabbit-anti-GRB2 and rabbit-anti-P75NTR(D4B3) XP® mAb (Cell Signaling Technology), rabbit anti-Phospho-TrkB (Abcam, phosphor Y705, #ab52191), PrP mAb anti-6D11(Santa Cruz, sc-58581). Horseradish Peroxidase (HRP)-conjugated goat anti- mouse or rabbit IgG (#31430 or #31460) were purchased from Thermo. DAPI (D1306) was from Invitrogen. Protease inhibitor Cocktail set III (539134) and Proteinase K (124568) were from Merck. Enhanced ChemoLuminescence (ECL) system (NEL103E001EA) was from PerkinElmer.

### 2.3. Preparation of brain homogenates

Three Chinese golden hamsters inoculated intracerebrally with hamster-adapted scrapie agent 263K and three normal hamsters were enrolled in this study. The detailed procedure of animal assay was described elsewhere (Shi et al., 2015; Gao et al., 2016). Briefly, 2 μl of 10% brain homogenates of scrapie agent 263K-infected hamsters were injected into the parietal lobes of two weeks old female hamsters at a depth of 4–5 mm. The incubation time was evaluated as 70.5±4.93 days. At the end of the clinical phase, the animals were euthanized using ether and their brains were surgically removed. Meanwhile, the brain samples of the agent 263K-infected hamsters collected on the 20th, 40th and 60th day post inoculation (dpi) were included as well. For dynamic assays, the brain samples collected at the terminal stage of infection was nominated as the samples of 80 dpi. The brains of three hamsters intracerebrally injected with 2 μl of physiological saline sacrificed at the 80th days post-injection were used as healthy controls. The brains were removed surgically, immediately dissected, then frozen and stored at −80°C until use. 10% (w/v) brain homogenates were prepared based on the protocol described previously (Yao et al., 2005). Briefly, brain tissues were homogenized in lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10 mM Tris, pH 7.5) containing a mixture of protease inhibitors. The tissue debris was removed with low speed centrifugation at 2000 *g* for 10 min and the supernatants were collected for further study.

### 2.4. Cell culture

Scrapie agent Chandler-infected cell line SMB-S15 and its normal cell line SMB-PS were obtained from Roslin Institute, UK. Cell line SMB-S15 was established originally by culture from the brain of a scrapie strain Chandler-infected mouse, showing mesodermal origin (Haig and Clarke, 1971). Cultured cells were washed twice with PBS, and were harvested with cold lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 10 mM Tris, 0.5% Na deoxycholate, pH 7.4)containing Cocktail Set III (Calbiochem, USA). The lysates were placed on the ice for 30 min After centrifugation at 10,000 *g* 4°C for 15 min, the supernatants were collected and the protein concentrations were determined by a BCA method. The prepared lysates were frozen at −80°C for further experiments.

### 2.5. Western blots

Cellular lysates or brain homogenates were separated by 10% or 15% SDS-PAGE and electro-transferred onto a nitrocellulose (NC) membrane (Whatman, USA). All protein extracts were quantified using BCA reagent (Calbiochem, USA) before resolution with SDS-PAGE and electrotransfer to nitrocellulose membranes. Membranes were blocked with 5% (w/v) bovine serum albumin (BSA) in 1×Tris-buffered saline containing 0.1% Tween 20 (TBST) at room temperature (RT) for 2 h and probed with individual primary antibodies at 4°C overnight. After washing with TBST, membranes were subsequently incubated with different HRP-conjugated secondary antibodies and reactive signals were visualized using an ECL kit.

### 2.6. Immunohistochemical staining

Brain tissue was fixed in 10% buffered formalin solution and paraffin sections (5 μm in thickness) were prepared routinely. After washing with PBS for three times, tissue slices were quenched