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Dysfunction of mitochondrial dynamics in the brains of scrapie-infected mice

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ABSTRACT

Mitochondrial dysfunction is a common and prominent feature of many neurodegenerative diseases, including prion diseases; it is induced by oxidative stress in scrapie-infected animal models. In previous studies, we found swelling and dysfunction of mitochondria in the brains of scrapie-infected mice compared to brains of controls, but the mechanisms underlying mitochondrial dysfunction remain unclear. To examine whether the dysregulation of mitochondrial proteins is related to the mitochondrial dysfunction associated with prion disease, we investigated the expression patterns of mitochondrial fusion and fission proteins in the brains of ME7 prion-infected mice. Immunoblot analysis revealed that Mfn1 was up-regulated in both whole brain and specific brain regions, including the cerebral cortex and hippocampus, of ME7-infected mice compared to controls. Additionally, expression levels of Fis1 and Mfn2 were elevated in the hippocampus and the striatum, respectively, of the ME7-infected brain. In contrast, Dlp1 expression was significantly reduced in the hippocampus in the ME7-infected brain, particularly in the cytosolic fraction. Finally, we observed abnormal mitochondrial enlargement and histopathological change in the hippocampus of the ME7-infected brain. These observations suggest that the mitochondrial dysfunction, which is presumably caused by the dysregulation of mitochondrial fusion and fission proteins, may contribute to the neuropathological changes associated with prion disease.

1. Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders that affect both humans and animals [1,2]. Scrapie is a prototypical prion disease that affects sheep and goats. Clinically, scrapie is characterized by a long latent period, progressive ataxia, tremor, wasting and ultimately death [3]. Many scrapie strains have been isolated from sheep and goats and used to examine not only the range of various pathogenesis pathways but also the neuropathological mechanisms induced by prion disease [4]. Typical features of the disease include the formation of spongiform vacuoles and astrocytosis, the formation of amyloid plaques in some cases and neuronal loss in the brain [5,6]. A key event in prion disease is the conformational misfolding of the endogenously expressed cellular prion protein (PrP^{C}) into the scrapie form of the pathogenic prion protein (PrP^{Sc}) [5].

A number of recent studies have demonstrated that mitochondria are dynamic organelles that continually undergo fission and fusion with one another [7–9]. Mitochondria can change in number and morphology within a cell during development, throughout the cell cycle and when challenged with various cytotoxic stimuli [9]. In mammals, the key molecules involved in mitochondrial fission are dynamin-like protein 1 (Dlp1, also referred to as Drp1) and fission 1 (Fis1). The process opposing fission, i.e., mitochondrial fusion, is controlled in mammalian cells by mitofusin 1 (Mfn1), mitofusin 2 (Mfn2) and optic atrophy 1 (Opa1) [7]. The sizes, shapes and interconnectivities of mitochondria are determined by their fusion and fission [9]. It has been suggested that the mitochondrial defects associated with Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) may result, at least in part, from a disruption of the fusion and fission mechanisms of mitochondria [8,9]. A recent study reported that the expression of Dlp1 is decreased and that mitochondria are

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abnormally elongated in the fibroblasts of AD patients and in neuronal cell lines overexpressing amyloid precursor protein (APP) [10]. The study suggests that APP causes an imbalance between mitochondrial fusion and fission that results in an abnormal distribution of mitochondria, which in turn contributes to mitochondrial and neuronal dysfunction. Several observations suggest a link between mitochondrial dysfunction and PD. Pink1 and parkin, which are PD-related genes, promote mitochondrial fission and inhibit mitochondrial fusion in *Drosophila* [11]. Additionally, HD research has focused on mitochondrial dysfunction. A mouse model of HD exhibits early defects in respiration and ATP production [12]. Moreover, mutant huntingtin seems to disrupt mitochondrial Ca^{2+} buffering [13] and to cause mitochondrial ultrastructural changes in the lymphoblasts of HD patients [14]. Previous studies reported that dysfunction and enlargement of the mitochondria occur by oxidative stress in animal models of prion disease [15,16]. However, the underlying mechanism responsible for this mitochondrial dysfunction remains unclear.

In the present study, we investigated the mitochondrial fusion and fission proteins that may be involved in the mitochondrial dysfunction observed in prion disease. We show that mitochondrial fusion and fission proteins are differentially modulated in the terminal stage of an experimental mouse model of prion disease and that this modulation may contribute to the morphological damage and reduction in number of mitochondria in infected neuronal cells.

2. Materials and methods

2.1. Antibodies

The following monoclonal and polyclonal antibodies were used: mouse monoclonal anti-PrP (3F10) [17], mouse monoclonal anti-COX IV (Abcam), goat polyclonal anti-enolase (Santa Cruz), mouse monoclonal to β-actin (Sigma–Aldrich), mouse monoclonal anti-Opa1 and mouse monoclonal anti-Dlp1 (BD Transduction Laboratories), mouse monoclonal anti-Mfn2, chicken polyclonal anti-Mfn1 (Novus Biologicals) and rabbit polyclonal anti-Fis1 (Santa Cruz).

2.2. Animals and scrapie strains

Six-week-old C57BL/6 mice were obtained from the Central Laboratory Animal (Republic of Korea) and divided into two groups: one group was infected with the ME7 scrapie strain, and the other included age-matched controls. The ME7 scrapie strain was kindly provided by Dr. Alan Dickinson (Neuropathogenesis Unit, Edinburgh, UK). This scrapie strain was maintained by serial intracerebral passages of brain homogenate from a terminally affected mouse. The mice were intracerebrally inoculated with 30 μl of 1% (w/v) brain homogenate in 0.01 M phosphate-buffered saline (PBS, pH 7.4) from either a normal brain or an ME7-infected C57BL/6 mouse brain at the terminal stage of the disease. When the clinical signs of prion disease were evident in the terminal stage (160 days postinoculation, dpi), the mice were sacrificed.

2.3. Western blot analysis

Whole brains or hippocampal regions were homogenized gently in 10-fold greater volumes (w/v) of 50 mM Tris–HCl (pH 7.4) containing 150 mM NaCl, 1 mM EDTA, 0.25% Na-deoxycholate, 1% NP-40 and protease inhibitor cocktails (Roche). The protein concentration was determined using the BCA assay (Thermo Scientific). The homogenates were treated with Proteinase K (PK) at a concentration of 50 μg/ml. Equal amounts of protein (10–50 μg in all assays) were separated by SDS–PAGE using 10%, 12% or 15% acrylamide gels and then transferred to nitrocellulose membranes (Thermo Scientific). After blocking with 5% skim milk for 1 h, the membranes were incubated with the individual antibodies overnight at 4 °C and then incubated with horseradish peroxidase-conjugated secondary antibody in Tris-buffered saline with 0.05% Tween-20 (TBST) containing 5% skim milk for 1 h at room temperature. The blots were visualized with the SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific). The expression levels of each protein were quantified using ImageJ software (NIH).

2.4. Transmission electron microscopy (TEM)

The animals were perfused with 0.1 M PBS (pH 7.4) containing 4% paraformaldehyde and 2.5% glutaraldehyde under deep anesthesia with 16.5% urethane. The brains were removed and fixed in the 0.1 M PBS fixative that was used for perfusion. The bilateral hippocampal regions were trimmed into small pieces immediately after their surgical removal and kept in the fixative for 2 h at 4 °C. Post-fixation was performed in 0.1 M PBS with 1% osmium tetroxide followed by dehydration through a graded ethanol series and embedding in Epon 812. Ultra-thin sections (75 nm) prepared by using an ultramicrotome (RMC MTXL) were stained with uranyl acetate and lead citrate and were subsequently observed with a transmission electron microscope (JEM-1011, JEOL). The numbers of total and damaged mitochondria were counted in the hippocampal neurons in the control and ME7-infected brains and then calculated based on fifteen arbitrarily selected hippocampal neurons. The sizes of the mitochondria were measured under high magnification (×50,000) using TEM (iTEM, Olympus Soft Imaging Solutions, GmbH), and the values were calculated as the means ± SDs of the lengths and widths of thirty arbitrarily selected mitochondria from each group. Statistical analyses of the numbers and sizes of the mitochondria were performed using Jandel SigmaStat software (V 3.5).

2.5. Statistical analyses

The compared values were calculated as the mean ± SD of three brains from each group, and statistical significance was determined using Student's *t*-tests.

3. Results

3.1. Imbalanced expression of mitochondrial fusion and fission proteins in infected brains

First, we identified deposits of PK-resistant PrP^{Sc} in ME7-infected brains in the end stage; the normal PrP^{C} proteins were completely degraded by the PK treatment, as shown in Fig. 1. To determine whether the mitochondrial fusion and fission proteins are affected by prion infection, their expression patterns were investigated in whole brains of the control and ME7-infected mice at the end stage of disease (160 dpi) (Fig. 2). Of the mitochondrial fusion and fission proteins (fusion proteins: Opa1, Mfn1 and Mfn2; fission proteins: Dlp1 and Fis1), Western blot analysis revealed that only Mfn1 was differentially expressed in the infected whole brains compared to the age-matched control brains (Fig. 2A and B). It has previously been reported that the hippocampal regions are more severely damaged than any other regions in brains infected with the ME7 scrapie strain [18]. Thus, to compare the expression levels of the mitochondrial fusion and fission proteins in various brain regions, we dissected both the control and ME7-infected brains into the following regions: cerebral cortex, hippocampus, cerebellum, striatum and brainstem. We found that the levels of Mfn1 and Fis1 were significantly

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