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Differentially expressed genes in iron-induced prion protein conversion

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ABSTRACT

The conversion of the cellular prion protein (PrP^C) to the protease-resistant isoform is the key event in chronic neurodegenerative diseases, including transmissible spongiform encephalopathies (TSEs). Increased iron in prion-related disease has been observed due to the prion protein-ferritin complex. Additionally, the accumulation and conversion of recombinant PrP (rPrP) is specifically derived from Fe(III) but not Fe(II). Fe(III)-mediated PK-resistant PrP (PrP^{res}) conversion occurs within a complex cellular environment rather than via direct contact between rPrP and Fe(III). In this study, differentially expressed genes correlated with prion degeneration by Fe(III) were identified using Affymetrix microarrays. Following Fe(III) treatment, 97 genes were differentially expressed, including 85 upregulated genes and 12 downregulated genes (≥1.5-fold change in expression). However, Fe(II) treatment produced moderate alterations in gene expression without inducing dramatic alterations in gene expression profiles. Moreover, functional grouping of identified genes indicated that the differentially regulated genes were highly associated with cell growth, cell maintenance, and intra- and extracellular transport. These findings showed that Fe(III) may influence the expression of genes involved in PrP folding by redox mechanisms. The identification of genes with altered expression patterns in neural cells may provide insights into PrP conversion mechanisms during the development and progression of prion-related diseases.

1. Introduction

Fatal neurodegenerative prion disorders result from protein misfolding [1]. Prion protein is the key component of prion diseases, also known as transmissible spongiform encephalopathies (TSEs), including scrapie in sheep, chronic wasting disease (CWD) in elk and deer, bovine spongiform encephalopathy (BSE) in cattle, and variant Creutzfeldt-Jakob disease (vCJD) in humans. During the progression of prior disease, a conformational change in normal prion protein (PrP^C) to the disease-specific PrP isoform (PrP^{Sc}) causes resistance to protease digestion and insolubility in water, yielding a protease-resistant PrP (PrP^{res}). PrP^C has several functional roles, and studies have proposed a relationship between PrP^C and metal homeostasis, particularly for iron and copper [2,3]. Oxidative stress, including the redox process, has been observed in response to the imbalance in metal homeostasis in the diseased brain [4,5]. Two oxidative states of iron are implicated in prion diseases [4,6–8]. In our previous study, the conversion and intracellular accumulation of recombinant PrP (rPrP) were specifically derived from Fe(III) rather than Fe(II). Furthermore, Fe(III) does not induce PrP^{res} formation by coming in direct contact with rPrP. Fe(III)-mediated rPrP conversion to PrP^{res} requires a complex cellular environment [9]. Although the pathogenic mechanisms of neurodegeneration, particularly the generation of the infectious isoform of PrP (PrP^{Sc}), have been studied extensively [3,5,10,11], factors for the acquisition of protease resistance are not completely defined. Microarray analysis has performed to obtain information regarding the factors involved in disease development and progression, and several studies have been conducted to identify gene expression changes related to prion disease [12–14]. In this study, we identified differentially expressed genes correlated with prion degeneration dependent on the oxidative states of iron using Affymetrix microarrays and total RNA samples extracted from cells treated with iron and rPrP. Gene ontology (GO) annotations were

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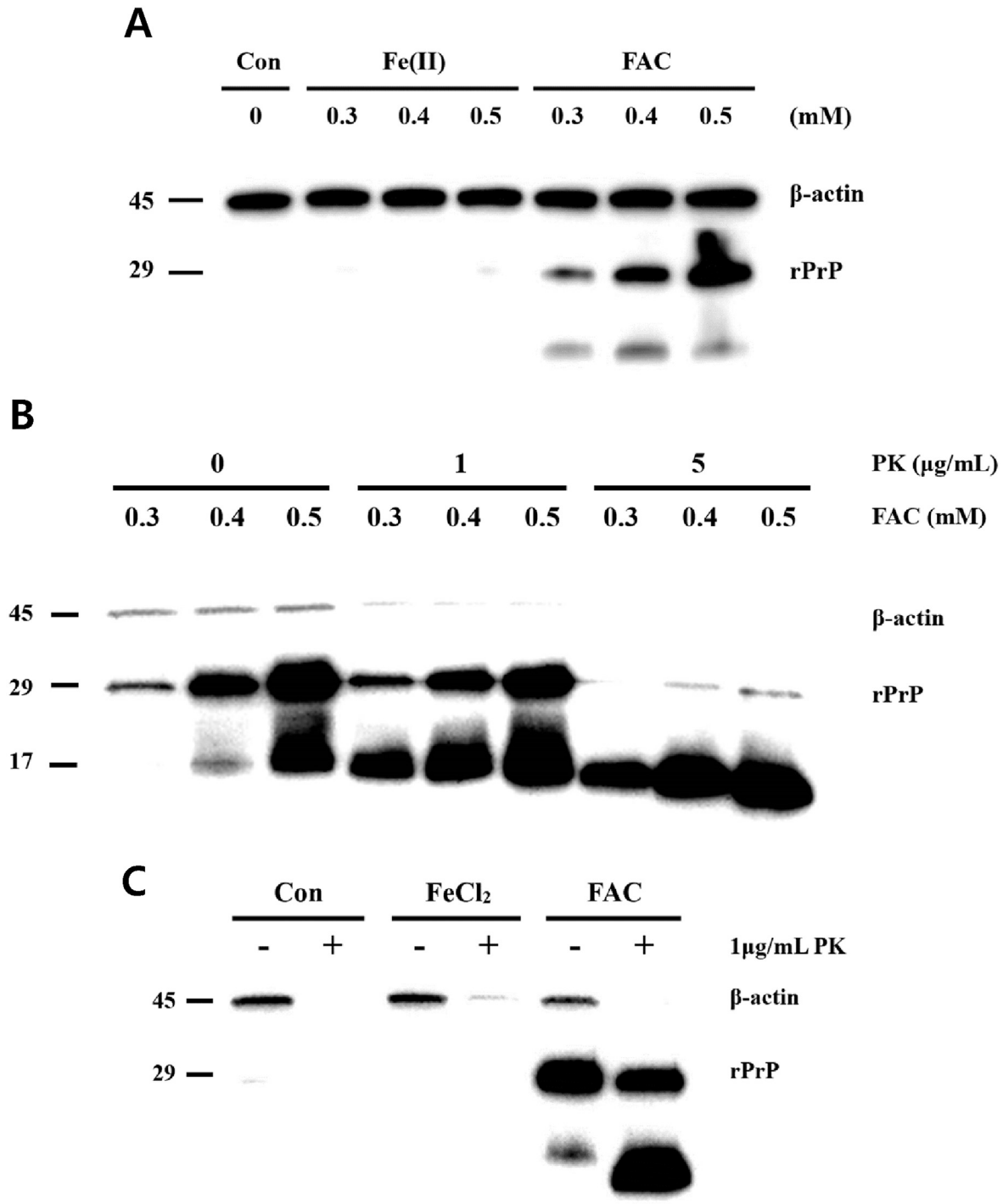

Fig. 1. Accumulation of PK-resistant internalized rPrP. (A) Cells were treated with 0.3–0.5 mM of either FeCl2 or FAC for 24 h. Subsequently, 0.6 μM rPrP was added for another 24 h. The internalized rPrP was detected with the 1E4 antibody. Internalized rPrP accumulated in an iron concentration-dependent manner. (B) PK concentrationdependent digestion of internalized rPrP induced by different concentrations of FAC. (C) The levels of internalized rPrP accumulated under either FeCl2-or FAC-treated conditions were compared according to PK treatment (+: with PK treatment; −: without PK treatment).

performed to determine the functional groupings of differentially regulated genes.

2. Materials and methods

2.1. Generation of recombinant protein

Bovine rPrP was cloned in a pET23 vector and expressed in *Escherichia coli* BL21 (DE3). Induction was performed with 1 mM isopropyl β-D-1-thiogalactopyranosid (IPTG) for 16 h before cloning. Cells were resuspended and sonicated in cold phosphate-buffered saline (PBS) containing protease inhibitor cocktail and 5 mM EDTA. Samples were centrifuged at 50,000 × *g* for 30 min; solubilized with 20 mM sodium phosphate (pH 7.4), 0.5 M NaCl, 20 mM imidazole, and 6 M guanidine-HCl; and then sonicated briefly. The solubilized proteins were collected again by