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Human doppel protein possesses inhibitory activity to superoxide dismutase

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ABSTRACT

Doppel gene (PRND) is a paralogue of the mammalian prion gene (PRNP). As prion protein expression might aid cellular resistance to oxidative stress by influencing the activity of superoxide dismutase (SOD), cellular changes in the human PRND-transfected HEK293 cells were also investigated. The SOD activity of transfectants with doppel (Dpl) expression was significantly lower than that of parental cells at 48 h after transfection, while prion protein expression increased cellular SOD activity. The SOD activity in amino-terminal truncation of Dpl transfectant was also decreased. The glycosylphosphatidylinositol (GPI) anchor site at the carboxyl-terminus of Dpl appeared to be essential for the repression.

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KEYWORDS

Doppel;
PRND;
Prion;
Superoxide dismutase;
Glycosylphosphatidylinositol.

INTRODUCTION

Similarly to the prion protein, Dpl is thought as a GPI anchored protein and is attached to the cell membrane by the anchor. The GPI anchor signal is composed of a stretch of hydrophobic amino acid residues in the carboxyl terminal region of GPI anchored protein. The normal function of Dpl remains to be established, however, its localization on the cell surface via a GPI anchor would be consistent with roles in cell adhesion and recognition, ligand uptake, as well as transmembrane signaling[1,2]. It has been reported that wild type prion protein plays a neuroprotective role against apoptosis induced by serum deprivation, and the octapeptide repeat region of prion protein plays an essential role in regulating apoptosis through the activation of SOD[3,4]. Although there have been several reports regarding the association between prion and SOD, it remains unclear whether prion elicits SOD activity itself or indirectly by activating cellular SOD[5]. The Dpl shares significant biochemical and structural homology with the normal prion protein, however, the Dpl can induce Purkinje cell death by nonapoptotic mechanisms, even in the absence of proapoptic Bax protein[6]. Strikingly, the neurotoxicity of Dpl is counteracted and prevented by the normal prion protein. Antagonistic functions for them were thus indicated from genetic and biochemical evidences.

Accordingly, we assumed that Dpl expression might disturb the SOD activity on the cells. This had prompted us to investigate whether the Dpl expression had the inhibitory activity against SOD or not.

RESULTS AND DISCUSSION

In the present study, we developed a mammalian

expression system for a truncated form of human Dpl protein with or without a GPI anchor site driven by CMV promoter in transiently transfected HEK293 cells to investigate the cellular changes of the SOD activity[7] (Figure 1). The SOD activity was determined by using the superoxide dismutase assay kit-WST (Dojindo, Japan) according to the protocol of the manufacturer, which utilizes a tetrazolium salt (WST-1) for the detection of superoxide radicals generated by xanthine oxidase. Each transfected cells were lysed and mixed with the radical detector, and the catalysis of SOD was then initiated by the addition of xanthine oxidase. The absorbances were read at 450 nm for WST-formazan, and SOD activities of the samples were calculated using a serially-diluted SOD standard reference curve. At least four individual experiments were performed. As shown in Figure 2, the SOD activity was increased in the human prion expressing cells and decreased in the human Dpl expressing cells compared to the parental HEK293 cells. On the other hand, no decrease in SOD activity was observed in the carboxyl truncated Dpl which had no

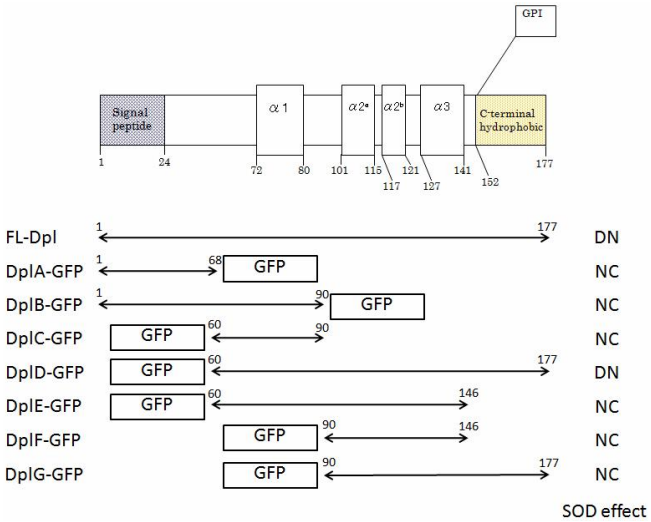

Figure 1 : Schematic representation of full length (FL) prion, FL-Dpl and deletion mutant proteins used in Figure 2. The corresponding PRND sequence to the indicated portion of Dpl proteins (DplA-GFP and DplB-GFP) or (DplC-GFP, DplD-GFP, DplE-GFP, DplF-GFP, and DplG-GFP) were inserted into either pcDNA3.1CT-GFP or pcDNA3.1NT-GFP plasmid, respectively. These plasmids were introduced into HEK293 cells together with a constant amount of PSV-GAL, followed by incubation for 48 hr. The amount of GFP protein expression was then measured by western blot and normalized as to expression of ERK1 and beta-galactosidase activity as equal levels of protein loading (not shown).

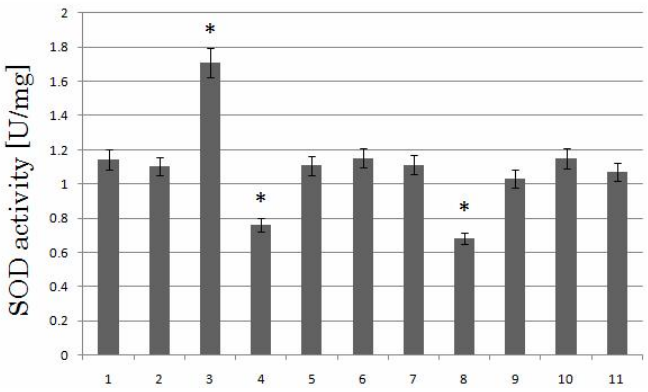

Figure 2 : Analysis of SOD activity in parental HEK293 cells and HEK293 cells transfected with various types of FL-prion, FL-Dpl, and Dpl mutant fused to GFP gene as shown in Figure 1: 1, HEK293; 2, GFP; 3, FL-prion; 4, FL-Dpl; 5, DplA-GFP; 6, DplB-GFP; 7, DplC-GFP; 8, DplD-GFP; 9, DplE-GFP; 10, DplF-GFP; 11, DplG-GFP. A SOD assay kit-WST (Dojindo, Japan) was used for the quantification of SOD activity. Cell lysate at 48 h after transfection in ice-cold RIPA buffer was assayed and compared with 1 unit of bovine erythrocyte SOD activity. The SOD activity was estimated using a standard curve of SOD activity on absorbance at 450 nm. The SOD activity was expressed as units/mg protein. Data are results from four independent experiments and are expressed as mean ± SD. * P<0.01 compared with HEK293 cells.

GPI anchor site. The viability of transfectants expressing exogenous human Dpl was not decreased while the capacities for cellular function against anti-SOD activity (data not shown).To summarize the results, both GPI anchor site and alpha helix domains were important for the SOD inhibition.

Prion protein is aslo a GPI anchored membrane protein that is thought to play a role in protecting against oxidative stress, and this protection is mediated by affecting SOD. This has been reported in recombinant, mutant, and normal prion protein in vitro and in vivo[4,8]. It may be a stress sensor that is sensitive to copper at octapeptide repeats, and it is able to initiate a signal transduction process acting on the antioxidant systems[9]. In addition, the level of the total SOD activity was correlated to the level of prion protein expressed[10]. These suggested that Dpl protein expression might also regulate SOD activity because of its structural similarity to prion protein. However, the present study demonstrated that GPI anchored Dpl might downregulate cellular SOD activity but GPI anchorless Dpl might not. These findings suggest that Dpl regulates cellular SOD activity via the

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GPI anchorage. Our results agree with other reports that the transfectant expressing both endo- and exogenous prion protein showed higher SOD activity on cells[11]. This suggests that the expression of the prion aids in the cellular response of the donor cells to oxidative stress.

It has been reported that cellular function of Dpl is counteracted by the normal prion protein[12]. To get some clues and evaluate how Dpl expression could lead to an inhibition of SOD activity, we investigated the effects of Dpl expression on signaling pathways known to be important for cell apoptosis. Western blot analyses for phopho-p38, phospho-Bad, phosphs-AKT and DFF45 were performed to investigate their protein levels. While the GFP-tagged Dpl and prion were clearly expressed in the HEK293 cells, there was almost no change in the level of the examined proteins involved in the apoptosis pathway (Figure 3). Neither FL-Dpl nor several truncated Dpl proteins expression also resulted in any changes in the examined protein level (data not shown). The lack of changes in the protein level was surprising, however, these results suggested that Dpl expression by itself did not affect the intra-cellular apoptotic signaling pathway. One possible reason is that the Dpl inhibit SOD on the cell surface, hence superoxide might injury the cell expressing Dpl only when superoxide is actually produced outside of the cells.

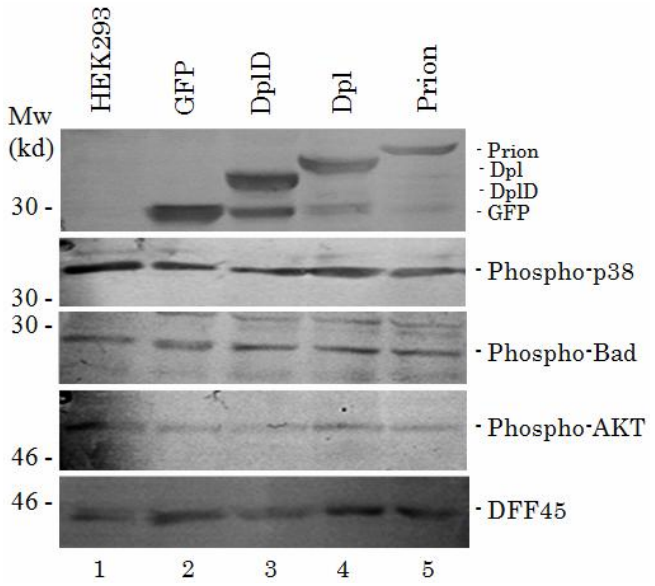

Figure 3 : Intra-cellular changes of proteins involved in apoptosis signaling pathway by Dpl and prion protein expression. The indicated protein levels were examined by western blot. Transfected HEK293 cells expressing GFP, prion, Dpl fused to GFP were harvested and analyzed in western blot developed with indicated antibodies (BD Biosciences) including anti-GFP antibody (SantaCruz). Western blot with anti-Erk1antibody was also checked as equal levels of protein loading (data not shown).

Numerous studies have demonstrated that various GPI anchor proteins, which are post-translationally modified, can affect the localization of these proteins in the plasma membrane or the cell wall. The GPI anchored proteins are structurally and functionally diverse and play vital roles in numerous biological processes, however, the biological functions of the GPI anchor have not yet to be elucidated at molecular level[13]. GPI anchored prion is important in the amplification and spread of prion infectivity from cell to cell[14], because membrane anchoring induced refolding of the prion protein to intermolecular beta-sheets[15]. Finally, it will be of particular interest to explore whether the Dpl share functional aspects with the SOD related molecules and might interact each other, as the prion protein does.

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COMPETING INTERESTS STATEMENT

The authors declared that no conflict of interest exists.

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