The analysis of CD44 in spinal cord of ALS model mice

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INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is an adult onset neurodegenerative disease that selectively affects motoneurons in the brain and spinal cord. Dominant mutations in the gene encoding Cu²⁺/Zn²⁺ superoxide dismutase 1 (SOD1) are the most prominent known causes of familial ALS. However, the mechanism of the neurodegenerative process and disease progression in ALS remains unclear.

The CD44 is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. This is expressed in a large number of mammalian cell types. This protein participates in a wide variety of cellular functions.

But there is no report but the only previous one about SOD1 and CD44 until now. The one is described about peripheral nerves (Gorlewicz A, et al. Neurobiol Dis. 2009). There is no report in central nervous system (CNS). So, my aim of this study is to analyze the relationship between SOD1 and CD44 in CNS.

MATERIALS and METHODS:

Animals: We used two types of animals in this study. The one is wild Type mouse: C57BL/6J (WT), and the other is ALS model mouse: B6.Cg-Tg(SOD1-G93A)1Jut/J (SOD1G93A).

We performed various kind of experiments as below and compared the data from each animal group in postnatal 18 weeks (onset of the disease) and 24 weeks (end stage).

1) Western blot analysis: Equal mount of protein from the lumbar spinal cord were subjected to 10% SDS-PAGE followed by electrophotting onto a BVDF membrane. After blocking, the anti-mouse CD44 monoclonal antibody was applied at a concentration of 1:1000 overnight, then the secondary antibody was applied for 30 minutes at RT. Bound antibodies were visualized with the ECL Plus Western blotting detection kit.

2) Real-time PCR: Total RNA was extracted from lumbar spinal cord with the RNeasy kit. Real-time PCR were done with Max300SP. SYBR-green was used as instruction of manufacture. The expression levels of CD44 were normalized by the copy number of GAPDH.

3) Immunohistochemistry: Ten-micrometer-thick sections were cut at the level of the enlargement in lumbar spinal cord. The sections were treated with a general immunohistochemistry protocol. After preincubation with bovine serum albumin, the anti-mouse CD44 monoclonal antibody was applied at a concentration of 1:500 overnight, then the secondary antibody was applied for 1 hour. We observe the specimen with a confocal microscope.

4) Primary culture and Western blot analysis: We performed primary culture of mixed glia from whole brain of P1 WT mice. Then, isolation of microglia or astrocyte is conducted 3 weeks after seeding. Astrocyes was stimulated by IFN-γ+TNF-α or bFGF for 72 hours, and microglia by LPS+IFN-γ for 72 hours. Then, CD44 expression of the each kind of cells was analyzed by Western Blotting by the same protocol described above.

RESULTS and DISCUSSION:

1) Western blot analysis: CD44 was observed as a band about 85kDa. In SOD1G93A mice, the expression of CD44 is higher than WT mice. The older mice become, the more remarkable the accession of CD44 becomes in SOD1G93A mice. But, in WT mice, the expression of CD44 is a little and constant over time. (Fig.1)

2) Real-time PCR: There is very remarkable increase of CD44 in mRNA level in SOD1G93A mice. (P<0.05, Mann-Whitney U test) (Fig.2).

So, the accession of CD44 in SOD1G93A mice is confirmed in both protein and mRNA level. Now, what is the localization of the upregulated CD44? What is the source of this increase?

3) Immunohistochemistry (confocal image): In SOD1G93A mice, a part of activated microglia may express CD44 on them (white arrow in Fig.3a). And about astrocyte, the expression of CD44 is clearly seen on them in SOD1G93A mice. (white arrow in Fig.3b)

So, the source of upregulated CD44 is mainly astrocyte, and partially microglia.

4) Primary culture and Western blot analysis: In microglia, the expression of CD44 is little without stimulation, but it is remarkably increased by stimulation. In astrocyte, the expression of CD44 is seen without stimulation, but the increase of expression is seen by stimulation of IFN-γ+TNF-α and bFGF. (Data not shown)

CONCLUSION:

In lumbar spinal cord of SOD1G93A mice, CD44 is upregulated than WT mice in vivo. And the source of upregulated CD44 is mainly astrocyte, and partially microglia. The increase of expression of CD44 is also seen in primary culture of astrocyte or microglia by stimulation in vitro. CD44 may have an important role on inflammation of CNS, and it may be one of proteins associated with the pathology of ALS.