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Research Report

The distribution of the seizure-related gene 6 (Sez-6) protein during postnatal development of the mouse forebrain suggests multiple functions for this protein: An analysis using a new antibody

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ABSTRACT

The seizure-related gene 6 (*Sez-6*) encodes a transmembrane protein that is expressed in neuronal cells. A *Sez-6*-deficient mouse exhibits impaired spatial memory, motor deficits, and decreased anxiety levels. To understand the function of Sez-6 during the postnatal development of the forebrain, the spatiotemporal pattern of distribution of the Sez-6 protein was immunohistochemically analyzed using a new anti-Sez-6 antibody. Western blot analysis confirmed the specificity of this new antibody, and showed that the content of the Sez-6 protein in the cerebral cortex was highest during the neonatal period and decreased gradually thereafter. Immunohistochemical analysis revealed that Sez-6 immunoreactivity (IR) was detected in various brain regions, such as the hippocampus, cerebral cortex, piriform cortex, striatum, lateral amygdala, and olfactory tubercle. The expression patterns of Sez-6 in these brain regions was divided into three groups: i) in the cerebral cortex, hippocampus, and lateral amygdala, moderate-to-strong Sez-6 IR was detected in the first postnatal week and decreased gradually thereafter; ii) Sez-6 IR was not observed during the neonatal period in the striatum and the intensity of the signal increased gradually toward adulthood; and iii) strong Sez-6 IR was observed in the olfactory tubercle, regardless of the developmental stage. Furthermore, Sez-6 IR was detected in dendrites of hippocampal and cortical pyramidal neurons neonatally, whereas it localized around the soma after postnatal day 10. These spatiotemporal alterations of the regional and intracellular distribution of the Sez-6 protein suggest multiple functions for this protein during the postnatal development of the forebrain.

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1. Introduction

The seizure-related gene 6 (*Sez-6*) encodes a transmembrane protein that contains a threonine-rich domain, five short consensus repeats, two CUB (Clr/Cls, urinary EGF, and bone morphogenic protein)-like domains, a transmembrane domain, and a short cytoplasmic domain (Shimizu-Nishikawa et al., 1995b). *Sez-6* was originally identified as a gene with

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Abbreviations: ABC, avidin–biotin–peroxidase complex; CA, cornu ammonis; IgG, immunoglobulin G; IR, immunoreactivity; P, postnatal day; PBS, phosphate-buffered saline; PTZ, pentylenetetrazol; PVDF, polyvinylidine fluoride; Sez-6, seizure-related gene 6

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an mRNA that is upregulated in the cortex after the administration of the convulsant drug pentylenetetrazole (PTZ) (Shimizu-Nishikawa et al., 1995a). Sez-6 mRNA is expressed strongly in the cortical plate of the fetal brain (Kim et al., 2002). In the adult mouse brain, the cerebral cortex moderately expresses Sez-6 mRNA, whereas a strong level of Sez-6 mRNA is detected in the hippocampus, piriform cortex, and olfactory tubercle (Herbst and Nicklin, 1997). These reports suggest the developmental regulation of the expression of Sez-6 mRNA and imply that *Sez-6* plays critical roles in the development of the forebrain and in cognitive function in the adult brain. *Sez-6*-null mice display impaired spatial memory, motor deficits, and decreased anxiety (Gunnersen et al., 2007). Interestingly, pyramidal neurons in the cerebral cortex of *Sez-6*-deficient mice exhibit an increased number of dendritic branches and reduced excitatory synaptic connectivity in the cerebral cortex. The synaptotropic hypothesis states that the elaboration of dendritic arborization is modulated by synaptic inputs (Vaughn, 1989). *In-vivo* time-lapse imaging revealed that enhanced visual activity promotes the growth of dendritic arbors in optic tectal cells in *Xenopus* tadpoles (Sin et al., 2002). AMPA-receptor-mediated transmission enhances dendritic arbor growth by stabilizing branches (Haas et al., 2006). However, the discrepancy in Sez-6 expression patterns between fetal and adult brains, as described above, implies the spatiotemporal regulation of Sez-6 distribution during the postnatal development of the forebrain. Hence, detailed analysis of the expression of the Sez-6 protein during postnatal development would promote the understanding of its functions. In this paper, we report the pattern of expression of Sez-6 from postnatal day (P) 0 to adulthood (10 months) using immunohistochemistry with a new antibody raised against a recombinant Sez-6 protein. Moderate-to-strong Sez-6 immunoreactivity (IR) was observed in the cerebral cortex, hippocampus, and lateral amygdala in the neonatal period, and decreased gradually thereafter. In contrast, the striatum did not show Sez-6 IR until P10, and it gradually increased afterward. Further, Sez-6 IR was detected in dendrites of hippocampal and cortical neurons at P0, whereas it localized around the somatic body after P10. Such developmental alteration of the regional and intracellular distribution of Sez-6 suggests multiple roles for the Sez-6 protein.

2. Results

2.1. *Characterization of the anti-Sez-6 antibody*

To investigate the distribution of the Sez-6 protein in the developing brain, we raised an anti-Sez-6 antibody against a recombinant Sez-6 protein. The antibody detected proteins of 160 and 190 kDa in a cell lysate from Neuro2a cells after transfection of a cDNA encoding type II Sez-6 (Fig. 1A). Interestingly, Western blot analysis detected only the 190 kDa protein in the conditioned medium, even though the membrane-bound type of Sez-6 was transiently expressed in

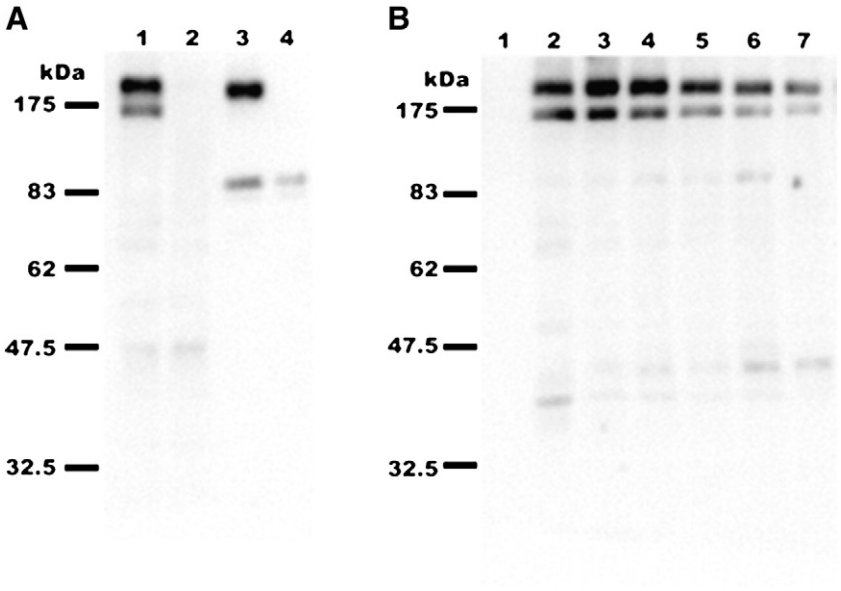

Fig. 1 – Western blot analysis using a new anti-Sez-6 antibody. (A) Specificity of the antibody. The anti-Sez-6 antibody recognized proteins of 160 and 190 kDa in a cell lysate (lane 1) of Neuro2a cells that expressed type II Sez-6 transiently. Only the 190 kDa protein was detected in the conditioned medium (lane 3). Neither the 160 kDa nor 190 kDa proteins were detected in the cell lysate or in the conditioned medium from mock-transfected cells (lanes 2 and 4). Lane 1, cell lysate of Sez-6-expressing Neuro2a cells; lane 2, cell lysate of mock-transfected cells; lane 3, conditioned medium of Sez-6-expressing Neuro2a cells; lane 4, conditioned medium of mock-transfected cells. (B) Temporal changes in the expression of the Sez-6 protein in the mouse forebrain. Thirty micrograms of membrane protein fractions from P0 to 8-week-old mouse brains were separated in 10% SDS-polyacrylamide gel electrophoresis under non-reducing conditions and blotted onto a PVDF membrane. The anti-Sez-6 antibody detected proteins of 160 and 190 kDa. The intensity of the signal appeared to be strongest at P6 and P10. Lane 1, neuro2a cell lysate as a negative control; Lane 2, P0; lane 3, P6; lane 4, P10; lane 5, P14; lane 6, P16; lane 7, postnatal week 8.

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