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Research Report

Abnormal expression and spatiotemporal change of Slit2 in neurons and astrocytes in temporal lobe epileptic foci: A study of epileptic patients and experimental animals

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ABSTRACT

Repellent guidance molecules provide targeting information to outgrowing axons along predetermined pathways during development. These molecules may also play a role in synaptic reorganization in the adult brain and thereby promote epileptogenesis. Our aim was to investigate the expression of Slit2, one of repellent guidance molecules, in temporal lobe epileptic foci from epileptic patients and experimental animals. Thirty-five temporal neocortex tissue samples from patients with intractable temporal lobe epilepsy (TLE) and fifteen histological normal temporal lobes from controls were selected. Fifty-four Sprague–Dawley rats were divided randomly into six groups, including five groups with epilepsy induced by lithium–pilocarpine administration and one control group. Temporal lobe tissue samples were taken from rats at 1, 7, 14, 30, and 60 days post-seizure and from controls. Expression of Slit2 was assessed by immunohistochemistry, immunofluorescence, and Western blot analysis. Slit2 was mainly expressed in neurons in human controls and in both neurons and astrocytes in TLE patients. Slit2 expression was significantly higher in TLE patients as compared with the controls. Slit2-positive cells were mainly neurons in the rat temporal lobe tissues of the control group, the acute period group, and the latent period group, while the Slit2-positive cells were mainly astrocytes in chronic phase. Compared with controls, Slit2 expression in animals in the TLE group gradually decreased from days 1 to 14 post-seizure, but then increased over the levels seen in controls, to peak levels at days 30 and 60. These results suggest that Slit2 may play an important role in the pathogenesis of TLE.

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Abbreviations: AEDs, antiepileptic drugs; MFS, Mossy fiber sprouting; OD, optical densities; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; CNS, central nervous system; SRS, spontaneous recurrent seizures; Tukey's HSD, Tukey's honestly significant differences; TBST, Tris-buffered saline–20% Tween 20; TLE, temporal lobe epilepsy

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1. Introduction

Epilepsy is a group of neurological conditions that are characterized by the occurrence of spontaneous recurrent seizures (SRS). Epidemiological data indicate that 20–40% of patients with newly diagnosed epilepsy will develop drug-refractory epilepsy (French, 2007). Temporal lobe epilepsy (TLE), which is a common form of focal epilepsy, is often refractory to anti-epileptic drugs. The exact mechanism responsible for the development of TLE is still unknown. Thus, an improved understanding of epilepsy development, or epileptogenesis, may facilitate the identification of molecular targets for therapeutic intervention.

The ability to transiently and chronically change the central nervous system (CNS) is a phenomenon referred to as plasticity (Scharfman, 2002). Pathological disorders with recurring episodes of excessive neural activity can lead to plastic changes including gliosis, axonal sprouting, synaptic reorganization, and remodeling of neuronal networks. The plastic changes that follow seizures may aggravate the seizure condition, leading to chronic epilepsy or TLE (Leite et al., 2005). Importantly, little is known about the molecular signals that underlie plasticity and TLE development.

Four major classes of secreted axon guidance molecules have been implicated in the control of axonal guidance and neuronal migration during CNS development: Slits, Netrins, Ephrins, and Semaphorins. These molecules were recently suggested to have important roles in pathological conditions of the nervous system (Yaron and Zheng, 2007). They are involved in CNS repair and regeneration after injury (Curinga and Smith, 2008) and may play a role in synaptic reorganization in the adult brain and thereby promote epileptogenesis (Barnes et al., 2003; Holtmaat et al., 2003). Slit proteins have emerged as important repulsive cues among the extracellular guidance cues. Slit proteins induce growth cone repulsion via modulation of a family of transmembrane receptors called roundabout (Robo), which transmit the signal further downstream. Slit–Robo interactions mediate repulsive cues on growth cones and axons during neural development (Brose et al., 1999). Three Slit proteins (Slit1–3) (Itoh et al., 1998) and four Robo proteins (Robo1, Robo2, Robo3/Rig-1, and Robo4) (Huminiecki et al., 2002; Kidd et al., 1998) have been identified in mammals. Slit2 is a member of the Slit family, which has previously been demonstrated to regulate axonal guidance, branching, and neural migration through interaction with Robo. Research on mice deficient in Slit2 has shown that when Slit2 is removed, axons tend to defasciculate (Bagri et al., 2002; Plump et al., 2002; Shu et al., 2003). A previous study has shown that expression of Slit2 is upregulated during postnatal development and remains elevated in adult neurons (Marillat et al., 2002). This upregulation suggests that Slit2 may be involved in synaptic plasticity. Studies on peripheral nerve injury in the rat model have shown that slit2 mRNA expression is downregulated in facial motoneurons after axotomy (Fujiwara et al., 2008) and upregulated when the continuity of the basal lamina tubes is disrupted (Tanno et al., 2005). Another study has shown that slit2 mRNA is upregulated in cells surrounding necrotic tissue after a CNS injury (Hagino et al., 2003). These results indicate that Slit2 may play a role in synaptic plasticity and regeneration after nerve injury, both in the peripheral nerve system and the CNS.

Considering the possible functions of Slit2 in the nervous system, we hypothesized that Slit2 may participate in the plastic changes that occur during TLE development. To evaluate this hypothesis, we characterized the expression of Slit2 in temporal lobe epileptic foci in TLE patients and experimental animals and investigated its importance in epilepsy.

2. Results

2.1. *Patients*

2.1.1. *Demographic and clinical characteristics of the epilepsy subjects*

The epilepsy patients had a mean age of 27.14±10.11 years and consisted of 17 men and 18 women. The mean duration of seizure recurrence was 11.03±7.43 years. The control group consisted of 8 men and 7 women. The control group had a mean age of 30.27±11.95 years. Statistical analysis showed that there were no significant differences in age or gender between the TLE patients and controls ($p>0.05$).

2.1.2. *Immunohistochemistry and immunofluorescence staining of Slit2 in the temporal neocortex of patients with intractable TLE*

In controls, faint and scattered immunoreactivity for Slit2 was observed, mainly in neurons (Fig. 1a). In the temporal neocortex of patients with intractable TLE, Slit2 was observed in both neurons and astrocytes (Fig. 1b, c). Moreover, double-labeled immunofluorescence showed that Slit2-positive cells (green) coexpressed with NSE (red) in control group (Fig. 2a–c). In TLE group, some Slit2-positive cells (green) and GFAP (red) were coexpressed in astrocytes while some single labeling of Slit2 was seen in neurons (Fig. 2d–f). The immunofluorescence results further indicated that Slit2 was mainly expressed in neurons in control group and expressed in both neurons and astrocytes in TLE group. No immunoreactivity was seen when staining of temporal tissue was performed in the absence of primary antibody. The number of Slit2-positive cells per 1000 cells in TLE patient samples was 0.587±0.057, and that in the control group was 0.267±0.061. Student's *t*-test showed that Slit2 expression was significantly higher in the temporal neocortex of the TLE group compared with the control group ($p<0.05$) (Fig. 4a).

2.1.3. *Evaluation of Slit2 expression in the temporal neocortex of TLE patients by Western blot analysis*

To confirm the increase in Slit2 expression observed by immunohistochemical staining of the TLE temporal neocortex, we evaluated Slit2 expression by Western blot analysis (Fig. 5a). Slit2 protein was detected as a band at about 200 kDa. Slit2 expression was strong in the temporal neocortex of TLE patients, whereas it was relatively weak in control samples. β-Actin (42 kDa) was used as a positive control. Slit2 expression was normalized by calculating the ratio of optical density (OD) of the bands for Slit2 and β-actin. Student's *t*-test was used for statistical analysis of the differences between the TLE group and control group. The OD ratio in the TLE group was 0.951±0.392, whereas that in the control group, it was 0.422±0.146 ($p<0.05$) (Fig. 5b).