Expression of FLRT2 in postnatal central nervous system development and after spinal cord injury

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論文題目

Expression of FLRT2 in postnatal central nervous system development and after spinal cord injury

（生後中枢神経系及び脊髄損傷後における FLRT2 の発現）

論文の内容の要旨

[Introduction]

Fibronectin and leucine-rich transmembrane (FLRT) proteins are necessary for various developmental processes and in pathological conditions. FLRT2 acts as a homophilic cell adhesion molecule, a heterophilic repulsive ligand of Unc5/Netrin receptors, and a synaptogenic molecule. Although the function of FLRT2 in regulating cortical migration at the late gestation stage has been analyzed, little is known about the expression pattern of FLRT2 during postnatal central nervous system (CNS) development and after mechanical injury. Therefore, I investigated the detailed expression pattern of FLRT2 in the various region of the brain of neonatal and adult mice, and after spinal cord injury.

[Materials and Methods]

The following experiments were approved by the animal experiment committee of Hamamatsu university School of Medicine (2019012). P0, P7, and adulthood (>6 weeks old) *Flrt2-LacZ* knock-in (KI) mice were used for *LacZ* staining. Sagittal and coronal sections were cut at a thickness of 100 μm on a vibratome after 4%PFA perfusion, and then stained at 37°C overnight for X-gal (5-bromo-4-chloro-3-indolyl-β-galactoside). Images of sections were acquired using a transmission light microscope. For immunohistochemistry experiments, $Flrt2^{lx/lx}$ mice were crossed with Nestin-Cre. Sagittal and coronal brain sections were prepared at a thickness of 20 μm using a cryostat. The goat anti-FLRT2, rabbit anti-PSD95, guinea pig anti-VGLUT1 and mouse anti-GFAP-Cy3 conjugated antibodies were used for immunostaining. Images of the sections were acquired with the TCS SP8 confocal microscope. For spinal cord injury, tenth thoracic vertebra (T10) was induced using an Infinite Horizons impactor with an impact force of 60 kdyn. Animals in the sham group were conducted to laminectomy alone. These animals were sacrificed at 3, 7, 14, and 28 days post-injury.

[Results]

At the early postnatal stage, FLRT2 expression was largely restricted to several regions of the striatum and deep layers of the cerebral cortex. In adulthood, FLRT2 expression was more prominent in the cerebral cortex, hippocampus, piriform cortex (PIR), nucleus of the lateral olfactory tract (NLOT), and ventral medial nucleus (VM) of the thalamus, but lower in the striatum. Notably, in the hippocampus, FLRT2 expression was confined

to the CA1 region and partly localized on pre- and post-synapses whereas only few expression was observed in CA3 and dentate gyrus (DG). Strong FLRT2-LacZ staining was observed around lesion sites 7 days after thoracic spinal cord injury. And expression of FLRT2, induced at the subacute phase, was localized on GFAP+ reactive astrocytes. Interestingly, the provocation of GFAP at 7 days after the injury was decreased in Nestin-Cre; *FLRT2*$^{lx/lx}$ conditional knock out (cKO) mice.

[Discussion]

These dynamic changes in FLRT2 expression may enable multiple FLRT2 functions, including cell adhesion, repulsion, and synapse formation in different regions during CNS development and FLRT2 serves as a repulsive guidance molecule, inhibits CNS regeneration, acts as an adhesive molecule, and contributes to form glial scar after spinal cord injury. The postnatal expression pattern of FLRT2 suggests that it is likely involved in the formation of various neural networks, such as the cortico-basal ganglia-thalamo-cortical loop, the dorsal tegmental nucleus (DTN) neural network, related to the sleep and awakening mechanism, and the development of the olfactory system. The glial component of the scar after spinal cord injury consists of reactive astrocytes, NG2+ oligodendrocyte precursors, and microglia in the penumbra. Reactive astrocytes rapidly proliferate and densely populate the area around the lesion core within 7–10 days after injury. Our results suggested that FLRT2 expressed on reactive astrocytes contributes to formation of glial scars as an adhesive molecule and induction of GFAP and inhibits axonal regeneration as a repulsive molecule. Thus, inhibiting FLRT2 function by applying neutralization antibodies may ameliorate scar formation after spinal cord injury and promotes CNS regeneration.

[Conclusion]

FLRT2 exhibits different expression patterns at various stages from the early postnatal stage to maturity, indicating that it plays various roles in the brain development. Especially, confined expression of FLRT2 in the CA1 localized on pre- and post-synapses were observed, whereas only few expression was observed in CA3 and DG. Temporal upregulation of FLRT2 level in astrocytes at the boundary of lesion sites after spinal cord injury regulates formation of glial scar. This study can provide new ideas for therapeutic approaches and the treatment of spinal cord injury by targeting FLRT2.