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SCIENCE DIRECT

Developmental Brain Research 154 (2005) 199–209

DEVELOPMENTAL BRAIN RESEARCH

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Research report

Distribution of RA175/TSLC1/SynCAM, a member of the immunoglobulin superfamily, in the developing nervous system

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Accepted 20 October 2004
Available online 28 December 2004

Abstract

RA175 is a new member of the immunoglobulin superfamily with *trans* interaction activity, and it plays a role as a tumor suppressor in lung carcinoma (TSLC1) and as a cell adhesion molecule promoting the formation of functional synapses (SynCAM). Little is known about the biological function of RA175/TSLC1/SynCAM neural network formation during neurogenesis. We examined the distribution and colocalization of the RA175/TSLC1/SynCAM protein with other members of the immunoglobulin superfamily such as NCAM, L1, and TAG-1 in the mouse developing nervous system. Consistent with the expression of *RA175/TSLC1/SynCAM* mRNA, the protein was localized in the brain neuroepithelium at embryonic day (E) 9.5, neural crest at E10.5, motor neurons at E10.5, and olfactory epithelium at E16.5. In contrast with its mRNA, the protein was intensely detected on the fasciculated axons in the floor plates, ventral root, and dorsal funiculus in the E10.5–11.5 spinal cord and colocalized with NCAM and L1 on the ventral root and dorsal funiculus and partly colocalized with TAG-1 on the commissural axons and dorsal funiculus. In the E13.5–15.5 brain, RA175/TSLC1/SynCAM colocalized with NCAM and L1 on the developing thalamocortical fibers from the internal capsule (IC) and partly colocalized with TAG-1 on the cortical efferent axons in the intermediate zone (IZ). RA175/TSLC1/SynCAM was localized on the axons of some of the cortical neurons cultured in vitro. Thus, in addition to cell adhesion activity in the neuroepithelium and the synapses, RA175/TSLC1/SynCAM may be involved in neuronal migration, axon growth, pathfinding, and fasciculation on the axons of differentiating neurons.

Theme: Development and regeneration
Topic: Axon guidance mechanisms and pathways

Keywords: Cell adhesion molecule; Axon growth; Tangential migration; SynCAM; RA175; TSLC1; TAG-1; L1; NCAM

1. Introduction

RA175 is a new member of the immunoglobulin superfamily that is preferentially expressed during retinoic acid (RA)-induced neuronal differentiation of P19 embryonal carcinoma (EC) cells [13,26]. RA175 is a membrane glycoprotein with significant amino acid sequence similarity to neuronal cell adhesion molecule (NCAM) and Nectin [39]. RA175 is a Ca^{++}-independent cell adhesion molecule with homophilic *trans*-cell adhesion activity [14].

TSLC1, a human tumor suppressor gene located on chromosome 11q23.2, is the human orthologue of *RA175* [16,18,22]. Defects in this gene promote the metastasis of lung carcinoma. In the developing lung epithelium, RA175/TSLC1 is preferentially localized in the lateral membrane of the polarized cells lining the lumen [14]. RA175/TSLC1 has been shown to be a cell adhesion molecule promoting the formation of functional synapses (SynCAM) [6]. The intracellular domain of RA175/TSLC1/SynCAM has sequence similarity to contactin-associated protein 2 (Caspr2), a member of the neurexin family [14,32]. These molecules contain the respective

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doi:10.1016/j.devbrainres.2004.10.015

short amino acid sequences, EWLT and EYFI, at their C-terminus, which serve as binding sites for the type II PDZ domain [4,5,29]. RA175/TSLC1/SynCAM interacts with calcium/calmodulin-dependent serine protein kinase (CASK) via EYFI at the presynaptic membrane to form functional synapses [6].

In the developing central nervous system, the neuroepithelium exhibits cell polarity, detaches from the basal membrane, and differentiates into neural precursor cells. Upon differentiation into neurons, these cells form axon and dendrite structures for synaptic interaction. In the developing spinal cord, the commissural cell fibers extend toward the ventral midline from the neurons located in the dorsal region of the spinal cord. In the cortex of the developing brain, the cortical neurons, born early to form the preplate, function as a scaffold for the assembly of the cortical architecture [1,9,23,25,27]. Other cortical neurons, born later, migrate radially and insert themselves into the preplate to generate the cortical plate, which splits the preplate into the marginal zone and the subplate [23,25]. Pioneer neurons derived from the preplate guide the thalamocortical afferent axons into the cortex and the cortical neurons of the cortical plates extend efferent axons toward their targets through intermediate zone (IZ) [1,9].

Members of the immunoglobulin superfamily with cell adhesion activity promote neuronal migration, axonal growth, fasciculation, pathfinding, and synaptic formation in the developing nervous system and are involved in the formation of neural networks [11]. NrCAM and TAG-1 (axonin), members of the immunoglobulin superfamily, are expressed on the commissural axons that outgrow ipsilaterally to the floor plate [34,35]. NCAM, SC1, and L1 (NgCAM), other members of the immunoglobulin superfamily, and TAG-1 are localized at the bundled axons in the floor plates and at the dorsal funiculus [7,12,24,36]. L1 and TAG-1 are localized on the axons in the corticofugal fiber system including the IZ and the thalamocortical pathway (TC), and the latter is involved in the tangential migration of interneurons from the medial ganglionic eminence (MGE) [10].

RA175 mRNA is expressed in the neuroepithelium during brain morphogenesis. Its expression increases in various regions of the brain, including the cortex, hippocampus, thalamus, and amygdala during neurogenesis, and then decreases before birth [39]. *RA175* mRNA is transiently expressed in the motor neurons, neural crest, and dorsal root ganglia (DRG) in the developing spinal cord. However, little is known about the involvement of RA175/TSLC1/SynCAM in the neuronal migration, axonal growth, fasciculation, and pathfinding during neural network formation.

To clarify the biological role of RA175/TSLC1/SynCAM in neural network formation during neurogenesis, we examined the distribution of RA175/TSLC1/SynCAM in the developing spinal cord and in the corticofugal fiber system including IZ and TC of the developing brain by in situ hybridization (ISH) and immunostaining.

2. Materials and methods

2.1. Immunohistochemical staining

Antibodies of RA175/TSLC1/SynCAM, antibodies against a peptide corresponding to the C-terminal region of RA175/TSLC1/SynCAM, were prepared as described previously [14] and used for the immunostaining of the RA175/TSLC1/SynCAM in mouse developing nervous system. The mouse embryos at embryonic day (E)9.5, E10.5, E11.5, E12.5 E14.5, E15.5, and E16.5 were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4 °C overnight and then soaked in 30% sucrose/PBS at 4 °C overnight and embedded in Optimal Cutting Temperature (OCT) compound (Sakura Finetec. Tokyo) and were frozen. Frozen sections (10 μm thick) of embryos were cut on a cryostat and attached to slides coated with Vectabond reagent (Vector Laboratories, Burlingame, CA) and incubated with rabbit anti-RA175/TSLC1/SynCAM and or rat anti-NCAM and anti-L1 (Chemicon International, Temecula, CA), mouse anti-TAG-1 (4D7, Developmental Studies Hybridoma Bank, Iowa City, IA) in PBS containing 0.1% skim milk and 0.1% Triton X-100 at 4 °C for 2 days as described previously.

Subcellular localization of RA175/TSLC1/SynCAM in the neurons was also examined by immunostaining. Neurons and astrocytes were isolated from the brain of E15.5 mouse embryos described previously [3] and cultured in Dulbecco's Modified Eagle's Medium (Sigma) containing 4500 mg/l glucose, 10% Fetal Bovine Serum in the presence or absence of 10 μM Ala-C for 10 days, respectively. Neurons and astrocytes were fixed at 4% paraformaldehyde and were immunostained with anti-RA175/TSLC1/SynCAM, mouse Tuj-1 (Tubulin β-III, Neuromics Antibodies Northfield, MN), mouse anti-GFAP, anti-Map-2 (Sigma, ST, Louis MO), and mouse anti-Tau (Upstate Biotechnology Lake Placid, NY).

Anti-RA175/TSLC1/SynCAM immunoreactivity was detected by FITC-labeled goat anti-rabbit IgG (Leinco Technologies, St. Louis, MO). Rat anti-NCAM and anti-L1 immunoreactivities were detected by goat anti-rat IgG Alexa Fluor 568 (Molecular Probes, Eugene, OR). Mouse anti-TAG-1, anti-Tuj-1, anti-GFAP, anti-Map-2, and anti-Tau immunoreactivities were detected by goat anti-mouse IgG Alexa Fluor 568 (Molecular Probes). Immunoreactivity was viewed using a confocal laser-scanning microscope (CSU-10, Yokogawa, Tokyo, Japan).

2.2. In situ hybridization (ISH)

The *Eco*RI fragment of *RA175/TSLC1/SynCAM* in the pGEM-T vector was subcloned into pBluescript SK(+)