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Expression of Semaphorin 4F in neurons and brain oligodendrocytes and the regulation of oligodendrocyte precursor migration in the optic nerve

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ABSTRACT

Semaphorins are secreted or membrane-anchored proteins that play critical roles in neural development and adult brain plasticity. Sema4F is a transmembrane semaphorin found on glutamatergic synapses, in which it is attached to the PSD-95-scaffolding protein. Here we further examined the expression of Sema4F by raising specific antibodies. We show that Sema4F protein is widely expressed by neurons during neural development and in the adult brain. We also demonstrate a preferential localization of this protein in postsynaptic dendrites. Moreover, Sema4F is expressed not only by neurons but also by oligodendrocyte precursors in the optic nerve and along the migratory pathways of oligodendroglial cells, and also by subsets of postnatal oligodendroglial cells in the brain. Finally, in vitro experiments demonstrate that endogenous Sema4F expressed by brain cells of oligodendroglial lineage regulates the outgrowth migration of oligodendrocyte precursors and promotes their differentiation. The present data extend our knowledge about the expression of Sema4F and uncover a novel function in the control of oligodendrocyte precursor migration in the developing brain.

Introduction

Semaphorins serve as axon guidance molecules – mostly repulsive – in neural tissue (Bagnard et al., 1998; Falk et al., 2005; Fenstermaker et al., 2004; He et al., 2002; Kantor et al., 2004; Pasterkamp and Kolodkin, 2003; Pasterkamp et al., 2003; Skaliora et al., 1998; Song et al., 1998). In addition, these molecules influence the migration of neurons (Kerjan et al., 2005; Marin and Rubenstein, 2003; Raper, 2000; Tamagnone and Comoglio, 2004) and glial cells (Cohen et al., 2003; Spassky et al., 2002; Taniguchi et al., 2009). Semaphorins are well characterized as brain circuit regulators but are also involved in the responses of the immune system (Kikutani and Kumanogoh, 2003) as well as in cardiovascular development (Kruger et al., 2005; Toyofuku et al., 2004), among other less characterized, organogenetic programs (reviewed in Roth et al., 2008).

While in vertebrates the most studied semaphorins are secreted proteins that diffuse over the extracellular matrix (class 3 semaphorins), several members are transmembrane (classes 4 to 6) or membrane-attached proteins (class 7) (Semaphorin Nomenclature Committee, 1999). When expressed on the surface of neurons, these semaphorins affect the formation of synapses during development, or plasticity events in the adult (Dityatev et al., 2008; Skaper et al., 2001). Various studies have addressed class 3 semaphorins and CNS lesions (Kaneko et al., 2006; Lee et al., 2010; Pasterkamp and Verhaagen, 2001). However, transmembrane counterparts may also be involved in these effects, especially class 4 semaphorins, which constitute the largest transmembrane subclass (Raper, 2000). In fact, Sema4D induces growth cone collapse of CNS axons (Giraudon et al., 2004; Swiercz et al., 2002), it is expressed by oligodendrocytes and it is upregulated after adult CNS lesion (Moreau-Fauvarque et al., 2003). In the Peripheral Nervous System (PNS), Sema4F is involved in the maintenance of Schwann cell–axon interaction through downregulation of the Ras/Raf/ERK pathway (Parrinello et al., 2008). Of note, some reports point to a double role of oligodendroglial cells as being synthesizers of and sensitive to multiple members of the semaphorin family (Cohen, 2005; Taniguchi et al., 2009).

Class 5 and class 6 semaphorins in the CNS have also been reported. Sema5A is specific of oligodendroglial lineage and it inhibits axon growth (Goldberg et al., 2004; Hilario et al., 2009; Kantor et al.,

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2004) as well as glioma cell motility via plexinA3 (Li and Lee, 2010). Class 6 semaphorins are potent inhibitors of axon outgrowth via plexins (Rünker et al., 2008; Suto et al., 2007; Tawarayama et al., 2010; Xu et al., 2000).

Semaphorin receptors are multimeric (Zhou et al., 2008). They always include a member of the single transmembrane-spanning proteins called plexins, which contain a conserved sema domain of inhibitory action, and an intracellular GTPase-activating (GAP) domain (Gherardi et al., 2004; Jackson and Eickholt, 2009; Oinuma et al., 2004). Concerning class 3 semaphorin receptors, these consist of plexins and neuropilins, the latter acting as co-receptors (reviewed in Kruger et al., 2005; Pasterkamp and Kolodkin, 2003). Ig-CAMs and other membrane-attached molecules contribute to the signaling mediated by diffusible semaphorins (reviewed in Zhou et al., 2008). Compelling evidence points to the proteolytic cleavage of membrane-associated semaphorins to generate soluble proteins that add further diversity to the signaling process (Chabbert-de Ponnat et al., 2005; Zhu et al., 2007). Recent studies performed on peripheral neurons demonstrate that transmembrane semaphorins interact with their receptors either in cis or in trans, thereby leading to differential responses (Haklai-Topper et al., 2010).

Class 4 semaphorins show an immunoglobulin-like domain next to their Sema domain and a short intracellular tail, which includes proline-rich sequences and a phosphorylation consensus sequence in the case of Sema4F (Encinas et al., 1999; Semaphorin Nomenclature Committee, 1999). This semaphorin is known to interact with specific scaffolding proteins such as PSD-95 in the CNS and is thus ascribed to neurons at glutamatergic synapses (Schultze et al., 2001). Here we describe the distribution of Sema4F in the developing as well as in the adult brain. We show that this molecule is expressed by neurons and oligodendrocytes; furthermore, we provide evidence of the participation of Sema4F in oligodendrocyte precursor migration.

Results

Sema4F transcript is widely expressed in mouse brain during development

To determine the expression pattern of Sema4F in mouse brain, we first studied its distribution by in situ hybridization (ISH) using a 1.6 kb-long RNA probe coding for the extracellular region of rat Sema4F (eSema4F). This probe, which was called We, was over 97% homologous to the mouse sequence (Encinas et al., 1999) (Fig. 1). Sema4F transcripts were widely expressed in most brain regions during development, from E14 onwards (Figs. 1A and M). Staining was particularly strong at E16-P0 (Figs. 1B, D, E, G, H and L). Expression remained high during postnatal development (Figs. 1N and O), and was still significant in adult mouse sections, although clearly decreased (Figs. 1C, F, I, J, K and P). The cerebellum, which was widely reactive at postnatal ages (Fig. 1O), remained strongly stained during adulthood, mostly in the granular layer, although the deep nuclei were also significantly stained (arrows in Fig. 1P). A Northern blot assay using total brain RNA showed a single transcript expressed at different ages, which was especially prominent at P0 (Fig. 2A).

At embryonic stages, mRNA expression was particularly high in the proliferative layers (e.g., ganglionic eminence at E14, Fig. 1A, neuroepithelium at E16, Fig. 1D) as well as in the anterior subventricular zone (aSVZ) at early postnatal stages (Fig. 1H). At postnatal ages, precursor cells from the aSVZ are known to migrate through the rostral migratory stream (RMS), which is stained in the adult (Fig. 1J), to the olfactory bulb (Fig. 1K), which is also rich in labeled cells in the adult. Most young postmitotic neurons also expressed Sema4F transcripts (cortical plate, Figs. 1A and D; early pyramidal and granular cells of the hippocampus, Fig. 1E). In the adult brain, Sema4F was expressed by many neuronal populations (see Table 1 for a detailed pattern). In addition to neurons, transcripts of this protein were also expressed in cells resembling glial cells (arrows in Figs. 1B and E; see also Fig. 5). For instance, the arrows in Figs. 1B and E point at the oligodendroglial precursor migratory pathway (OMP) of the hippocampus; the corpus callosum, devoid of neurons, is clearly labeled at P0 (Fig. 1G; see also Fig. 5). Labeling with the We probe indicates that Sema4F transcripts are widely expressed by neuronal precursors, glial cells and postmitotic neurons in the developing and adult brain.

Distribution of Sema4F protein in brain during development

To study the distribution of Sema4F, a rabbit antiserum was raised against an extracellular peptide distal to the Sema domain (residues 567 to 587 of murine Sema4F; Encinas et al., 1999). We purified the IgG fraction, which we named anti-4F, and used it for immunochemical studies. Anti-4F was previously tested for specificity in COS1 cells transfected with a Myc-tagged full-length Sema4F-coding expression vector (Fig. 2B). Similarly, Western blot (WB) against cell lysates from Myc-tagged Sema4F-transfected COS1 cells yielded prominent bands that were not detected in cells transfected with other transmembrane semaphorins (Fig. 2C). Finally, we show that our anti-4F antiserum immunoprecipitates Sema4F (Fig. 2D).

In WB assays, Sema4F was detected as a single band of about 150 kDa in neural tissue from E15 onwards; Sema4F was especially abundant in the cerebral cortex at perinatal stages, decreasing in this region from P5 onwards (Fig. 2E). In the cerebellum, it was especially abundant at postnatal stages. In the adulthood, the protein was detected mainly in the cerebellum, hippocampus and cerebral cortex (Fig. 2E). Interestingly, an additional band of around 75–80 kDa found exclusively in the adult brain was also observed (noted as ** in Fig. 2E).

We next performed an immunohistochemical analysis. Overall, the pattern of Sema4F immunoreactivity (IR) overlapped the patterns of mRNA expression; thus, most brain regions expressed Sema4F protein from E14 onwards (Figs. 3A and L). Expression was especially prominent in the olfactory bulb, cerebral cortex, hippocampus and brain stem. At E14, however, some proliferative layers expressing Sema4F mRNA did not show protein staining (compare GEm in Figs. 1A and 3A), thereby suggesting delayed protein expression. Immunolabelling was high between E16 and P0 (Figs. 3B, D, E, G, H and K). The hippocampus was strongly labeled at E18 (Figs. 3B and E) and the IR remained significant at P15 (Fig. 3F) and in the adult (Fig. 3C). The aSVZ was strongly stained around P0 (Fig. 3H), and was still reactive in the adult (Fig. 3I). The olfactory bulb was also stained along development and in the adult, mainly in the mitral cell layer and the glomerular layer (Fig. 3N). The RMS was equally stained (Fig. 3M). The CP was reactive at embryonic ages (Figs. 3A, B and D) and the cerebral cortex until the adulthood, IR being especially outstanding in the entorhinal cortex (Fig. 3J). The hypothalamus showed faint although widespread IR at embryonic ages (Fig. 3B), which was higher in particular areas at postnatal ages (Fig. 3O). Of note, a remarkable intensity was found near the arcuate and posterior hypothalamic nuclei at P10 (Fig. 3O; see also Fig. 5), an OMP region near the third ventricle, through which oligodendroglial precursors migrate at previous ages. In the hindbrain, several nerve nuclei were prominently stained, such as the oculomotor nucleus, the periaqueductal gray area, the colliculli, raphe and pararubral nuclei (Fig. 3K). The facial nucleus was also immunoreactive at P0 (Fig. 3L, pointed as 7nu) and most cell types of the cerebellum were reactive during development and also in the adult (Figs. 3P and Q). Sema4F expression was low although significant in adult neurons (Figs. 3C, F, J, N and Q) and, in the hippocampus, it was particularly relevant in the apical dendrites of CA1 pyramidal neurons (squares in Suppl. Fig. 1A) and around the granular cell bodies of the dentate gyrus (squares in Suppl. Fig. 1B).

To gain insight into the fine localization of Sema4F protein, we performed immunocytochemical analyses of hippocampal tissue at