The Distribution and Characterization of SHPS-1, that Binds Protein Tyrosine Phosphatase SHP-2, in the Human and Rat Brain

KAORU FUKUNAGA1,2, TAKASHI MATOZAKI2, YOSHIKANE HAYASHI1, YOSUKE FUJIOKA2, MASAHIRO TSUDA2, TOSHIYUKI TAKADA2, TETSUYA NOGUCHI2, MASATO KASUGA2 and HIROSHI ITO1

1First Department of Pathology and 2Second Department of Internal Medicine, Kobe University School of Medicine, Kusunokicho, Chuo-ku, Kobe 650-0017, Japan.

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ABSTRACT

SHPS-1 is a receptor-like glycoprotein that is tyrosine-phosphorylated and binds SHP-2, a Src homology 2 domain-containing protein tyrosine phosphatase, in response to various mitogens and cell adhesion. SHPS-1 is highly enriched in the brain with its molecular size of ~90 kDa. In contrast, it is also expressed in various other organs with its molecular size of 110 to 130 kDa, suggesting that the extents of glycosilation of SHPS-1 may be different among various tissues. Immunohistochemistry of human brain sections revealed that the most intense expression of SHPS-1 was observed in the neurons of hippocampus, inferior olive and putamen. High levels of SHPS-1 were also detected in cortical neurons and cerebellar Purkinje cells. In contrast, a weak or trace signals were observed in glia cells or white matter. Finally, the expression of SHPS-1 correlated with retinoic acid-induced neural differentiation of P19 cells. These results suggest that SHPS-1 may play important roles in a variety of neuronal cells.
highly expressed in the brain, although it is also expressed in various tissues (7, 12, 24). In addition, SHPS-1 is tyrosine-phosphorylated and forms a complex with SHP-2 in the brain (24). However, the precise role of SHPS-1, that forms complexes with SHP-2, in neuronal function remains largely unknown.

To better understand the physiological functions of SHPS-1 in the brain, we investigated the localization and characterization of SHPS-1 in the human and rat brain.

MATERIALS AND METHODS

Antibodies

The rabbit polyclonal antibodies to SHPS-1 (7, 36, 41) and to SHP-2 (21) was generated as described previously.

Immunoprecipitation and Immunoblot Analysis

For immunoblotting experiments, various organs of adult Wistar rats were homogenized with a motor-driven homogenizer (Kinmatica AG, Model RECO 61) in ice-cold lysis buffer [RIPA buffer: 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 10% glycerol containing 1 mM phenylmethylsulfonyl fluoride and 10 μg/mL aprotinin. The homogenates were then centrifuged at 10,000×g for 15 min at 4°C and the resultant supernatants were then solubilized in SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer. Gel electrophoresis and immunoblot analysis, with polyclonal antibodies to SHPS-1 or SHP-2 and an ECL detection kit (Amerham), were performed as described previously (22, 37).

Deglycosilation

Immunoprecipitation was first performed by incubating 1 mg of rat brain lysate prepared above with 5 μg of polyclonal antibodies to SHPS-1 prebound to Sepharose-protein G beads (Pharmacia) (20 μL beads) for 4 h at 4°C. The beads with immunoprecipitated proteins were washed twice with 1 mL of WG buffer [50 mM Hepes pH 7.6, 150 mM NaCl, 0.1% Triton X-100], and then incubated with or without 4 U/mL N-Glycosidase F (Boehringer Mannheim) for 4 h at 37°C. The samples were then analyzed by SDS-PAGE followed by immunoblotting with polyclonal antibodies to SHPS-1.

Immunohistochemistry

Brain sections from autopsied adults were prepared for immunohistological examination. Every case was routinely fixed with 10% buffered formalin and embedded in paraffin blocks. The expression and distribution of the SHPS-1 proteins in human brain sections was examined immunohistochemically with polyclonal antibodies to SHPS-1. Immunohistochemical staining was carried out using DAKO LSAB Kit, Peroxidase (Dakopatts, Copenhagen, Denmark) according to the manufacturer's instructions. The formalin fixed sections were deparaffinized in xylene (10 min, three times), then dehydrated in ethanol, and incubated with 3% (v/v) hydrogen peroxide for 20 min. The sections were then incubated with a blocking solution for 60 min and incubated with polyclonal antibodies to SHPS-1 (diluted 1:100 with 0.01 M phosphate-buffered saline (PBS) (pH 7.2), for 16 h at 4°C). After washing with 0.05 M Tris-HCl (pH 7.6), the sections were incubated with biotinylated anti-rabbit immunoglobulin, followed by incubation with horseradish peroxidase-conjugated streptavidin. Visualization was carried out with aminoethylcarbazole chromogen and hydrogen peroxide. The sections were counter stained with hematoxylin and mounted on slides.

Neural Differentiation of P19 Cells

P19 embryonal teratocarcinoma cells were cultured in alpha modified Eagle's medium supplemented with 10% (v/v) fetal calf serum under a humidified 5% (v/v) CO2 in air atmosphere at 37°C. In order to induce neural differentiation, the cells were cultured on bacterial grade dishes to form aggregates, termed embryoid bodies, for 4 days in the presence or the absence of 1 mM retinoic acid. Cells were then replaced on tissue culture grade dishes and cultured two additional days without retinoic acid. Thereafter, the culture medium was aspirated and cells were immediately washed with ice-cold PBS and frozen in liquid nitrogen. The cells were then lysed on ice in 1 mL of ice-cold lysis buffer [20 mM Tris-HCl (pH 7.6), 140 mM NaCl, 2.6 mM CaCl2, 1 mM MgCl2, 1% Nonidet P-40, 10% (v/v) glycerol] containing 1 mM phenylmethylsulfonyl fluoride and 1 mM sodium vanadate. The lysates were centrifuged at
10,000×g for 15 min at 4°C, and the resulting supernatants were subjected to immunoprecipitation and immunoblot analysis.

RESULTS

Distribution of SHPS-1 in Various Rat Tissues

We first determined the expressions of SHPS-1 proteins in various rat tissues by immunoblotting with polyclonal antibodies to SHPS-1. Immunoblotting of SHPS-1 showed that this protein is highly expressed in the brain with an apparent molecular weight of approximately 90 kDa (Fig. 1A). SHPS-1 is also highly expressed in the spleen and the moderate expression of SHPS-1 was observed in heart, lung and liver. In contrast to the brain, SHPS-1 proteins are expressed in various other organs with apparent molecular weights of approximately 110 to 130 kDa (Fig. 1A). Since SHPS-1 is a glycosilated protein (8), the differences in their molecular sizes might be due to differences in the extent of glycosilation of SHPS-1 proteins in various tissues. In fact, the treatments of lysates prepared from rat brain or spleen with N-glycosidase-F reduced the apparent molecular sizes of either SHPS-1 protein to ~60 kDa (Fig. 1B).

Distribution of SHPS-1 in the Rat Nervous System

We next examined the distribution of SHPS-1 in the rat nervous system. Total protein lysates prepared from various regions of the rat brain were immunoblotted with polyclonal antibodies to SHPS-1 (Fig. 2). The highest expression was observed in hippocampus and high expression was in cortical gray matter, striatum and cerebellum, while signals were weaker in tissues containing significant amounts of white matter such as substantia alba, thalamus and brain stem (Fig. 2).

Immunohistochemistry of SHPS-1 in Human Brain

The highest expression of SHPS-1 in the rat brain suggests that it has an important role in neuronal function. The distribution of SHPS-1 in mouse brain was examined previously (3, 4). Thus, we here determined the localization of SHPS-1 in the human brain by light microscopic immunohistochemistry (Fig. 3). In all regions of the brain, the intense immunoreactivity for SHPS-1 was found

Fig. 1 Expression of SHPS-1 and deglycosilation analysis in various rat tissues. A. Various rat tissues were homogenized in 0.32 M sucrose buffer, and 10 µg of each of the homogenates was subjected to SDS polyacrylamide gel electrophoresis followed by the immunoblotting with polyclonal antibodies to SHPS-1 (1:1000 dilution) B. Brain and spleen homogenates were treated with or without N-glycosidase-F as described in the Experimental Procedures, and resulting samples were subjected to SDS-PAGE followed by the immunoblotting with polyclonal antibodies to SHPS-1. Asterisk indicates a glycosilated SHPS-1.

Fig. 2 Distribution of SHPS-1 in the rat nervous system. Various regions of the rat brain system were separated under the microscope and homogenized in 0.32 M sucrose buffer and 10 µg of each protein were subjected to SDS-PAGE followed by the immunoblotting with polyclonal antibodies to SHPS-1.
in neurons, whereas weaker SHPS-1 immunoreactivity was detected in glial cells (Fig. 3). In most neurons, staining was found in the cytoplasm as well as cell membranes but not in nucleus. In some neurons with large dendritic processes, dendritic processes and axons also showed immunoreactivity. Most neurons in the cerebral cortex showed moderate to intense staining. Positive neurons were noted in all cortical layers of the frontal, parietal, temporal and occipital lobes (Fig. 3A, B). In the cerebellum, Purkinje cells displayed intense SHPS-1 immunoreactivity in the cytoplasm leaving a clear unstained nucleus, while molecular layer cells were faintly immunopositive and granule cells were almost immunonegative (Fig. 3C). In the hippocampus, pyramidal cells were immunoreactive in the CA1 to CA4 regions (Fig. 3D). The granule cells of the dentate gyrus also showed SHPS-1 immunoreactivity (Fig. 3D). In the medulla, intense immunoreactivity for SHPS-1 was found in the neurons of the inferior olivary nucleus (Fig. 3E), hypoglossal nucleus, and dorsal motor nucleus of the vagus nerve (data not shown). Thalamic neurons showed moderate immunoreactivity (Fig. 3F) and both medium and large neurons stained moderately in the putamen (data not shown).

Fig. 3 Immunohistochemical staining of adult human brain with polyclonal antibodies to SHPS-1. A, B. Immunohistochemical staining of cerebellar cortex was performed as described in the Experimental Procedures. A. (Magnification ×12.5) B. (Magnification ×200); C. Immunohistochemical staining of cerebellum. (Magnification ×200); D. Immunohistochemical staining of hippocampus. (Magnification ×12.5); E. Immunohistochemical staining of Thalamus. (Magnification ×200); F. Immunohistochemical staining of Inferior olive. (Magnification ×200).
Neuronal Differentiation Induces the Expression of SHPS-1

Since the SHPS-1 is highly expressed in most of neurons in human and rat adult brain, we next determined whether its expression is induced by neuronal differentiation of mouse P19 teratocarcinoma cells. SHPS-1 or SHPS-1 complexed with SHP-2 is hardly detected in undifferentiated P19 cells (Fig. 4). Retinoic acid is known to induce the neuronal differentiation of these cells (10, 28). When P19 cells were incubated with 1 μM retinoic acid for four days, the expression of SHPS-1 was markedly induced (Fig. 4), which was accompanied by morphological neuronal differentiation of these cells (data not shown). In addition, SHPS-1 complexed with SHP-2 was also induced by retinoic acid (Fig. 4).

DISCUSSION

Tyrosine phosphorylation has been indicated to be involved in signal transduction pathways in the central nervous system. High levels of tyrosine kinase (PTK) activity and several PTKs have been identified in different regions of the brain (31, 35). In addition, the receptor for nerve growth factor and other neurotrophic factors have been identified as various members of the Trk family of receptor PTKs (11, 13). Another PTK, Src, has been demonstrated to be highly expressed in the brain as well as platelets and to associate with plasma membranes through its NH₂-terminal region (5, 9). Although the precise function of Src kinase in the brain has not been elucidated, Src kinase is predominantly expressed in the neural growth cone membrane of fetal rat brain (16). However, very little is known about the control of tyrosine phosphorylation by PTPases in the central nervous system. Several receptor-type PTPases, such as DPTP10D (40, 44) or RPTPβ (15), have been demonstrated to be predominantly expressed in the brain. Contactin has recently been shown to be a ligand for RPTPβ, suggesting that the interaction of these two proteins leads neuronal development and differentiation (25). We (34) and others (26) have previously demonstrated that SHP-2 is highly expressed in the brain, although the physiological function of this PTPase in neural tissues is largely unknown. In the present study, we have demonstrated that SHPS-1 is highly expressed in human and rat brain. Although the SHPS-1 is ubiquitously expressed in various rat tissues, we found that the relative molecular size of brain SHPS-1 differs from those of other organs. In addition, the differences of the molecular sizes of SHPS-1 might be due to the distinct extents of glycosylation of SHPS-1 proteins. During the course of our experiments, the heterogeneity in terms of the molecular sizes of SHPS-1 in various tissues has also been demonstrated (29). The distinct glycosilation of SHPS-1 may indicates its distinct functions in various tissues. We have also demonstrated that the expression levels of SHPS-1 are heterogeneous in the various parts of brain. By the immunoblotting of lysates prepared from various parts of rat brain, similar levels of SHPS-1 were observed in cortical gray matter, striatum and cerebellum, while the signal was more intense in hippocampus and much weaker in white matter. Immunohistochemical analysis revealed that, in all regions of the human brain, the intense staining was detected in neurons, while weaker signals were detected in glia cells. In neuronal tissue, SHPS-1 is highly expressed in neurons of the hippocampus, cortical neurons and Purkinje cells of the cerebellum, to a lesser extent, in other glial cells. This distribution of SHPS-1 in the brain is almost identical to that of SHP-2 (34). We previously showed that SHP-2 was mostly associated with the synaptic membranes (34). This could be explained by the fact that SHP-2 preferentially binds to SHPS-1, a transmembrane protein, in the brain. We have also demonstrated
that the expression of SHPS-1 was accompanied by morphological neuronal differentiation of P19 cell. It remains unknown whether the expression of SHPS-1 induces neural differentiation, while SHPS-1 could be a useful surface marker for neural differentiation.

The physiological roles of SHPS-1, that forms a complex with SHP-2, in the brain remain to be determined. However, cerebral cortical neurons have been shown to extend their neurite on a plate coated with BIT/p84, a SHPS-1 homolog, and the antibodies to BIT/p84 specifically inhibited the effect (4, 29). This observation suggests that SHPS-1 may participate in cell-cell interaction of neuronal cells. The localization of SHPS-1, that binds SHP-2, in the hippocampus, thalamus, and piriform cortex is of great interest, because some Src family kinase have been reported to exist in similar regions in the brain (27, 32). In the steady state, the COOH-terminal tyrosine residue of a Src family PTK is phosphorylated to be enzymatically inactive (5). SHP-2 complexed with SHPS-1 might activate the PTK activity through dephosphorylation of the COOH-terminal tyrosine residue of a Src family kinase. A recent study with PTK inhibitors demonstrated that PTKs are required for the induction of long-term potentiation in the hippocampus (23), a well-characterized reversible change in synaptic transmission and also known as a model for memory formation (19). Thus, it is possible that SHP-2 complexed with SHPS-1 may regulate long-term potentiation and memory formation through dephosphorylation of its target such as Src family kinase. Further investigation is necessary to provide a better understanding of the physiological roles of SHPS-1 and SHP-2 in the brain.

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