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SAST124, A NOVEL SPLICE VARIANT OF SYNTROPHIN-ASSOCIATED SERINE/THREONINE KINASE (SAST), IS SPECIFICALLY LOCALIZED IN THE RESTRICTED BRAIN REGIONS

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Abstract—Syntrophin is an adaptor protein that binds signaling molecules to the dystrophin-associated protein complex, which connects extracellular matrix to intracellular cytoskeleton for construction and maintenance of the postsynaptic structures in the neuromuscular junction and the CNS. Among these signaling molecules, a family of microtubule-associated serine/threonine kinases has a unique structural feature with a serine/threonine kinase domain and a postsynaptic density protein-95/discs large/zona occludens-1 domain. In the present study, we identified syntrophin-associated serine/threonine kinase-124, a novel splice variant of the syntrophin-associated serine/threonine kinase which is a member of the microtubule-associated serine/threonine kinases family. Comparing to the original clone (syntrophin-associated serine/threonine kinase-170), syntrophin-associated serine/threonine kinase-124 is truncated just downstream of the postsynaptic density protein-95/discs large/zona occludens-1 domain. Using a monoclonal antibody specifically recognizing syntrophin-associated serine/threonine kinase-124, strong expression of the protein was observed in neurons of the subventricular zone and granule cells of the olfactory bulb, Islands of Calleja, hippocampal dentate gyrus and cerebellum. syntrophin-associated serine/threonine kinase-124 is selectively localized in the nuclei of neurons and distinct from syntrophin-associated serine/threonine kinase-170, which is interacting with syntrophin on the cell surface. Considering the tissue and subcellular distributions of syntrophin-associated serine/threonine kinase-124, it is suggested that syntrophin-associated serine/threonine kinase-124 may have functions in transcriptional regulation for the features commonly shared by these neurons. On the other hand, syntrophin-associated serine/threonine kinase-124 was also localized in glia-like cell bodies in the corpus callosum and fiber bundles in the spinal trigeminal and solitary tracts, suggesting syntrophin-associated serine/threonine kinase-124 may have other functions in these types of cells.

Key words: SAST170, MAST205, granule cell, PDZ domain, Zipro1 (RU49/Zfp38), subventricular zone (SVZ).

In the postsynaptic structure of the neuromuscular junction (NMJ), the dystrophin-associated protein complex (DAPC) is connecting extracellular matrix with intracellular F-actin cytoskeleton to maintain its synaptic structure. Function of this complex is important for the signal transmission at NMJ. In fact, Duchenne muscular dystrophy (DMD), a rapidly progressive lethal muscle disorder is caused by a mutation in dystrophin, one of major components in the complex (Hoffman et al., 1987; Bonilla et al., 1988; Brown and Hoffman, 1988). In addition, DMD patients also show moderate nonprogressive cognitive and memory impairment, suggesting importance of the DAPC function in the CNS (Lidov et al., 1996).

To reveal the function of DAPC, several major components of the complex have been extensively characterized. One of them, syntrophin is an adaptor molecule that connects signaling molecules, such as neuronal nitric oxide synthase (nNOS), stress-activated protein kinase-3 (SAPK3), receptor tyrosine phosphatase-like protein (ICA512), receptor subunit for neuregulin (erbB4) and growth factor receptor bound 2 (Grb2), to dystrophin and its complex (Brenman et al., 1996; Hasegawa et al., 1999; Garcia et al., 2000; Ort et al., 2000; Oak et al., 2001). Syntrophin also interacts with muscular Na^+-channels for integration of molecular signaling and neuronal activities in the NMJ (Gee et al., 1998; Schultz et al., 1998). Until now five isoforms, $\alpha1$, $\beta1$, $\beta2$, $\gamma1$ and $\gamma2$, have been identified in the family of syntrophins (Froehner et al., 1987; Adams et al., 1993; Piluso et al., 2000). The basic protein structure consisting of two pleckstrin homology (PH) domains, a postsynaptic density protein-95/discs large/zona occludens-1 (PDZ) domain and a syntrophin unique (SU) domain, is conserved among the isoforms. On the other hand, each isoform shows a unique tissue and subcellular distribution pattern (Froehner et al., 1987; Peters et al., 1994; Gorecki et al., 1997; Wakayama et al., 1997; Kramarcy et al., 2000). In neuromuscular junctions, $\alpha1$ syntrophin is localized in sarcolemma and throughout the junctional folds at the regions, while $\beta2$ syntrophin is essentially confined to the junctions. $\gamma1$ is specifically ex-

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Abbreviations: a.a., amino acids; CASK, calmodulin-dependent serine kinase; cDNA, complementary DNA; DAPC, dystrophin-associated protein complex; DAPI, 4,6-Diamidino-2-phenylindole; DGK-ζ, diacylglycerol kinase-ζ; DIV7, 7 days *in vitro*; DMD, Duchenne muscular dystrophy; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; Grb2, growth factor receptor bound 2; MAST, microtubule-associated serine/threonine kinases; MAST205, microtubule-associated serine/threonine kinase-205 kDa; mRNA, messenger RNA; NLS, nuclear localization signal; NMJ, neuromuscular junction; nNOS, neuronal nitric oxide synthase; PH, pleckstrin homology; PDZ, postsynaptic density protein-95/discs large/zona occludens-1; SAST, syntrophin-associated serine/threonine kinase; SGZ, subgranular zone; SU, syntrophin unique; SVZ, subventricular zone.

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pressed in the brain, while γ2 is distributed broadly in different tissues (Piluso et al., 2000). The unique distribution pattern of each isoform may be corresponding to its unique function in the particular regions.

Identification and characterization of signaling molecules binding to syntrophins are important for analysis of functions of syntrophins and their complex. Among syntrophin-binding proteins, a family of microtubule-associated serine/threonine kinases (MAST), including microtubule-associated serine/threonine kinase-205 kDa (MAST205) and syntrophin-associated serine/threonine kinase (SAST), has a unique structural feature with a serine/threonine kinase domain in their amino-terminal (N-terminal) region followed by a PDZ domain (Walden and Cowan, 1993; Lumeng et al., 1999). MAST205 is strongly expressed in the heart and testis, while SAST is specifically localized in the brain. The serine kinase activity associated with MAST205 immunoprecipitates was observed with *in vitro* assay system, suggesting their actual kinase functions *in vivo*. SAST can bind to the PDZ domains of β2 syntrophin and nNOS through its own PDZ domain region. SAST was enriched in the synaptosomal fraction and localized with syntrophin on the cell surface of cultured interneurons. In the brain, SAST was expressed widely in different regions including hippocampal pyramidal cells. These previous results showed that SAST might have important functions in the DAPC at postsynaptic sites with its serine/threonine kinase activity.

On the way to isolate delta2 glutamate receptor binding proteins, we identified a novel splice variant of the SAST gene transcript, SAST124. In this study, we produced a monoclonal antibody specifically recognizing the protein and revealed its distribution in brain.

EXPERIMENTAL PROCEDURES

Cloning of SAST124

For construction of a bait plasmid, the 57 amino acids (Gly^{951}-Ile^{1007}) of delta2 glutamate receptor C-terminus was fused to lexA protein on the plasmid pEG202. Using the bait plasmid (pEG202-delta2), a cDNA library constructed from rat adult whole brain messenger RNA (mRNA) (Cat. #DLR-102, Origene Technologies, Inc., Rockville, MD, USA) was screened with the DupLEX-A yeast two-hybrid system (Origene). The whole experiment was done according to the procedure provided by the company. Nucleotide sequences of the candidate genes were analyzed with the ABI PRISM 310 system (Applied Biosystems, Foster City, CA, USA).

For construction of a cerebellar cDNA library, mRNA was prepared from adult rat cerebellum (10 weeks old male, Wistar/ST) with the FastTrack 2.0 kit (Invitrogen Corporation, San Diego, CA, USA). After conversion to cDNA with the oligo-dT *Not*I primer-adaptor by reverse transcriptase (SuperScript II, GibcoBRL, Invitrogen Corporation), ligation into plasmid pSPORT1 was performed. The whole procedure was done using the SuperScript plasmid system (GibcoBRL) according to the method provided from the manufacturer. For screening of the cDNA library, the originally isolated fragment encoding the C-terminus of SAST124 (Asp^{618}-Val^{1117}) was re-cloned into pBluescriptII (Stratagene, La Jolla, CA, USA) for construction of a template plasmid, and then a [^{32}P] labeled RNA probe was synthesized by T7 RNA polymerase (Roche Diagnostics Corporation, Germany) using the template plasmid. After repeated screening of the cDNA library, eight candidate clones were isolated and their nucleotide sequences were determined with the ABI PRISM 310 system.

Production of monoclonal antibodies

For production of monoclonal antibodies, the C-terminal region of SAST124 (Asp^{618}-Phe^{1094}) was tagged with a histidine cluster (His x6) on pET30a(+) (Novagen, Inc., Madison, WI, USA) and the resultant fusion protein was purified with the His Trap kit (Amersham Pharmacia Biotech UK Ltd., UK) as an antigen. BALB/c mice were immunized for several times by intraperitoneal administration of the antigen (25–50 μg each) using RIBI adjuvant system (RIBI Immunochem Research, Inc., Hamilton, MT, USA) for 1.5 months and finally boosted with the same antigen (50 μg) in saline a month later. Three days after boosting, the spleen cells were corrected and fused with myeloma cells, X63-Ag8.653, in a medium of 50% polyethylene glycol 1500 (Roche Diagnostics). The fused cells were placed in hypoxanthine-aminopterin-thymidine growth medium containing 10% hybridoma cloning factor (Igen, Gaithersburg, MD, USA) and incubated in a chamber (10% CO_2) at 37 °C for several days. Hybridoma cell lines producing antibodies available for tissue immunostaining were isolated after screening by immunoblotting with the antigen and immunostaining of brain cryo-sections.

Immunohistochemistry

Under deep Nembutal (sodium pentobarbital) anesthesia, adult rats (4 weeks old male, Wistar/ST) were perfused through the left ventricle with physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH7.4). After removing from the skull and prolonged fixation for 3 h at 4 °C, the brains were cryo-protected in 30% buffered sucrose for several days until they sunk. Frozen sections of 35 μm thickness were cut on a freezing microtome, collected in PB, and immunostained as follows. The sections were pre-incubated for 2 h in blocking solution (3–10% normal goat serum in 0.1 M PB, pH 7.5, 0.2% Triton X-100). And then they were incubated in primary solution at concentration of 0.14 μg/ml for mAb#21 in the blocking solution at 4 °C for 60 h. The immunoreactive signals were visualized with an Alexa-488 conjugated secondary antibody (1:500, goat anti-mouse IgG; Molecular Probes, Inc., Eugene, OR, USA). The sections mounted on glass slides were examined with the LSM510 laser confocal microscope (Zeiss, Germany). No immunoreactivity was detected in control sections from the same brain blocks that were processed without the primary antibody.

Experiments on animals

All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. And all efforts were made to minimize the number of animals used and their suffering.

RESULTS

SAST124 has a unique amino acid sequence downstream of the PDZ-domain

To identify binding proteins to the delta2 glutamate receptor, 57 amino acid residues of the carboxyl terminal (C-terminal) of the receptor was fused to the lexA protein on a bait plasmid. Twelve candidate genes were identified after screening of a rat brain cDNA library with the yeast two-hybrid system. All of the candidates encoded proteins with one or two PDZ domains, suggesting that they bind to the C-terminal amino acid sequence of the delta2 receptor (-Thr-Ser-Ile) which belongs to the type I consensus sequence for interaction with a PDZ domain.

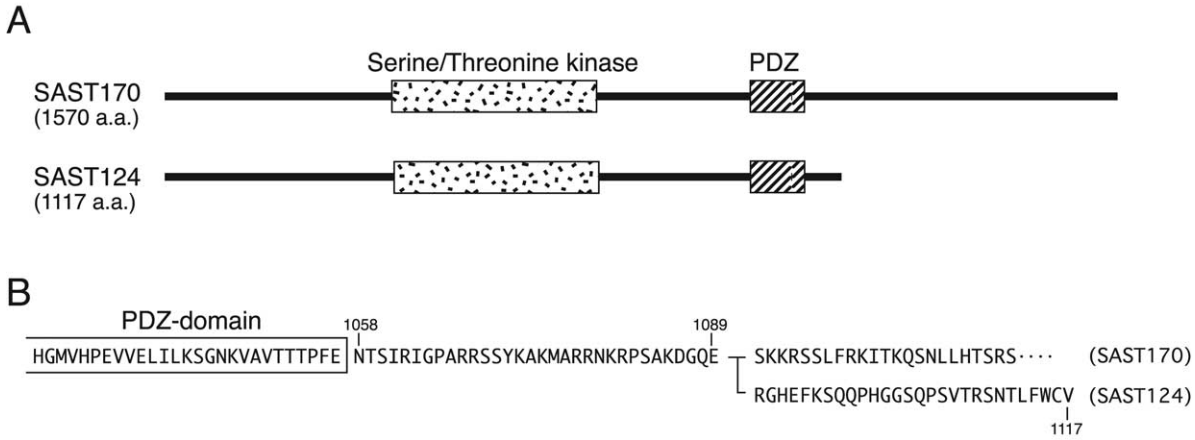

Fig. 1. Structure of SAST170 and SAST124 proteins. (A) Schematic diagrams of structure of SAST170 and SAST124. (B) Partial amino acid sequence of SAST170 and SAST124 around the splicing region. The entire amino acid sequence of SAST124 C-terminus is shown in the figure, while the downstream amino acid sequence is omitted for SAST170.

One of the candidate genes encoded a homologous protein with mouse SAST. The protein contained a 472 amino acids (a.a.) region which showed a sequence homology to Asp^{617}-Glu^{1088} of mouse SAST protein with 98.5% identity (465 a.a./472 a.a.), followed by a region of 28 a.a. non-homologous sequence. This originally isolated clone was used as a probe to identify the entire coding region of the gene. After screening a rat cerebellar cDNA library, two types of clones encoding different size proteins were isolated. The larger protein encoded by a clone had 1570 a.a. with 170 kDa estimated molecular weight. The amino acid sequence of the protein were 96.7% identical (1518 a.a./1570 a.a.) with mouse SAST protein, suggesting the protein is a counterpart of mouse SAST protein in rat. The other clone encoded a smaller protein with 1117 a.a. and its estimated molecular weight was 124 kDa. Up to the Glu^{1089}, the amino acid sequence of the smaller protein was identical to that of the larger one, whereas the sequence alignment downstream of the amino acid residue (Glu^{1089}) was completely different between these proteins (Fig. 1). The smaller protein had only 28 a.a. downstream of the residue (Glu^{1089}), while 481 a.a. were found in the larger one. The difference in amino acid sequence between the proteins suggested the smaller one is a novel splice variant of SAST gene products in rat. Because of the estimated molecular weight of each protein, the larger protein is called SAST170 and the smaller one is called SAST124. SAST124 was originally isolated as a binding protein to delta2 glutamate receptor, however, it was not expressed in cerebellar Purkinje cells, in which delta2 receptor is specifically expressed (Fig. 4G). Therefore, we did not do further experiments on the interaction between SAST124 and delta2 receptor.

SAST124 is specifically expressed in the brain

Using a set of primers which specifically enhances a part of SAST124 cDNA (223 bp) by PCR, expression of SAST124 was examined in several tissues in rat (Fig. 2). The PCR product of expected length was detected only in a reaction with cDNA from whole brain, while control PCR products of Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA were obtained in reactions with samples from all tissues. The results showed specific expression of SAST124 in the brain. In the previous study, expression of SAST170 was detected only in the brain with northern blot and western blot experiments (Lumeng et al., 1999). Combining together the present result, both of SAST170 and SAST124 are exclusively expressed in the brain.

A monoclonal antibody, mAb#21 specifically recognizes SAST124 and shows selective staining of neurons in the restricted brain regions

A part of the C-terminus of SAST124 (Asp^{618}-Phe^{1094}) tagged with a histidine cluster (His x6) was purified and injected into mice as an antigen for antibody production. After screening and cloning of hybridoma cells, 60 candidate clones were isolated and characterized for their antigenic recognition specificities.

One of the candidate monoclonal antibodies, mAb#21, could specifically recognize SAST124 protein in lysate from HEK293 cells transfected by SAST124 gene with immunoprecipitation and western blot analysis (Fig. 3B). mAb#21 recognize SAST170 protein only weakly in lysate of SAST170 trasfected cells, while the control antibody (mAb#14) reacted with both SAST170 and SAST124 proteins in the lysate. The same specificity of mAb#21 for SAST124 protein was observed in cerebellar lysate with western blot analysis (Fig. 3C). Using a series of truncated peptides of SAST124, the region (Gly^{965}-Glu^{1089}) close to the C-terminus of the protein was identified as the antigenic site for mAb#21 (Fig. 3A). Interestingly, the antigenic region for mAb#21 is commonly conserved in both of the splice valiant, SAST170 and SAST124. The difference in downstream of the region between these valiant may affect strongly on the structural conformation of the region to show selective reactivity with the antibody in the lysate and on the western blot. On the other hand, antigenic region of the control antibody (mAb#14), which can recognize both

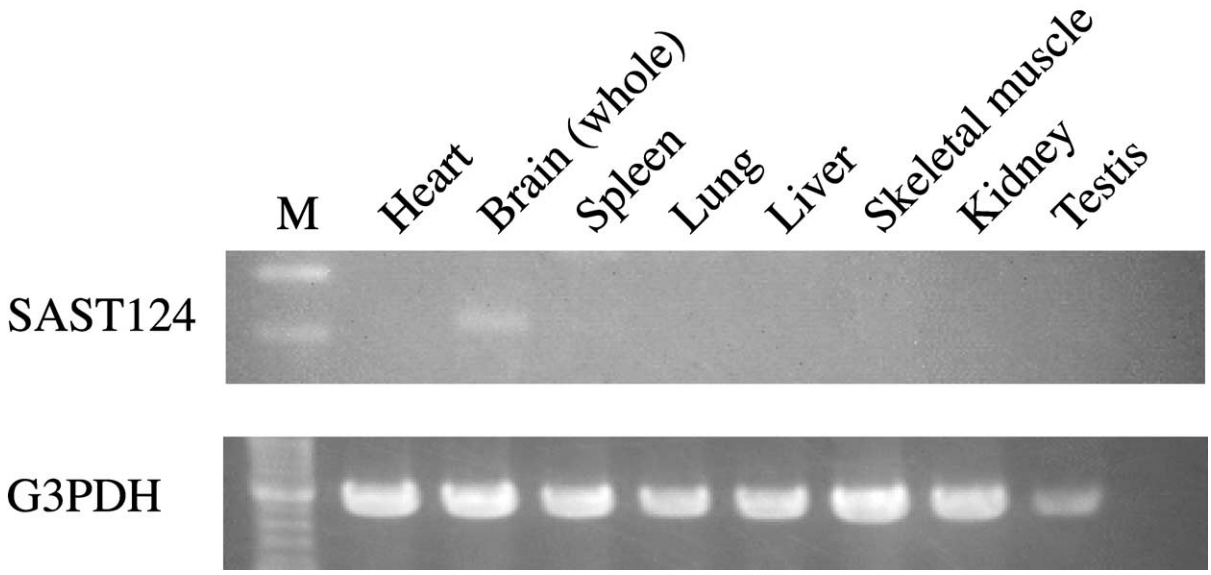

Fig. 2. Expression of SAST124 in various tissues. Expression of SAST124 was examined in various tissues of adult rat (8–12 weeks male, Sprague–Dawley) with RT-PCR method. The rat Multiple Tissue cDNA (MTC) panel (Cat. # K1429-1, Clontech Laboratories, Palo Alto, CA, USA), including same amount of cDNA prepared from heart, brain, spleen, lung, liver, skeletal muscle, kidney and testis, was used in the reaction. The left side column of both panels includes DNA marker fragments (100 bp ladder). A band with the expected length (223 bp) is detected only in the reaction with brain mRNA, whereas a band corresponding to the G3PDH PCR product (1 kb) is observed in all of the reactions.

valiant forms, is located between the kinase and PDZ domains in the protein (data not shown). This region is separated from the splicing site by the PDZ domain, therefore structural conformation of the region may be conserved in both variants to be recognized by mAb#14.

Using mAb#21, distribution of SAST124 was examined in the whole brain (Fig. 4). The strong immunostaining of neurons was found in only limited regions and cell types (Fig. 4A). In the olfactory bulb, granule cells of internal granular layer and periglomelular cells were immunopositive, but the mitral/tufted cells were not (Fig. 4B). The subependymal cells around the olfactory ventricle were strongly stained and the staining was continued to the subventricular zone (SVZ) of the lateral ventricle (Fig. 4C, F). In other forebrain regions, cells in the Islands of Calleja and dentate granular cells in the hippocampal region were moderately immunoreactive (Fig. 4D, E). In the cerebral cortex, layer II/III cells of granular retrosplenial area showed a weak immunoreactivity. In the cerebellum, granule cells were very strongly stained, but Purkinje cells and cells in the molecular layer did not show the immunoreactivity (Fig. 4G). Although a rather weak staining of neurons was also present extensively in most of the brain structures, it appeared to be a non-specific or a low affinity binding, because decreased concentration of the antibody resulted in a rapid disappearance of the staining from such regions. In neurons, the immunoreactants seemed to be mostly confined to the cell nucleus. This was confirmed in cultured cerebellar granule cells in which their nuclei were immunostained exclusively (Fig. 5).

In addition to the staining of neuronal cell bodies, the immunoreactivity was also found in many glia-like small cells in the corpus callosum as well as in a certain population of fiber bundles in the spinal trigeminal and solitary tracts (Fig. 4H, I). However, the immunoreactive cell bodies could not be detected in the nuclei of the tracts. In relation to the trigeminal nerve staining, the trigeminal ganglion was immunohistochemically examined, and some of the ganglion cells were found to be immunoreactive (Fig. 4J). In these cells, the perikaryal region rather than the nucleus contained the immunoreactants.

SAST124 is selectively localized in the nuclei of cerebellar granule neurons

Subcellular localization of SAST124 was examined in cultured cerebellar granule neurons. After fixation of 7 days *in vitro* (DIV7) culture, mAb#21 was applied to the cells, and signals were detected with Cy-3 conjugate secondary antibodies. Simultaneously, cellular nuclei in the culture were stained with 4,6-Diamidino-2-phenylindole (DAPI). In comparison with the staining pattern of DAPI in the culture, signals of mAb#21 were mostly localized in cellular nuclei of the granule cells (Fig. 5). In the stained nuclei, strong punctate staining pattern was observed in mAb#21 signals, suggesting localization of SAST124 at particular structures inside the nuclei (Fig. 5A).

DISCUSSION

In the present study, we identified SAST124, a novel splice variant of SAST, in the rat brain. Using the specific antibody for SAST124, it was shown that the protein is exclusively expressed in the restricted regions of the brain, especially in neurons of the SVZ and granule cells of the olfactory bulb, Islands of Calleja, hippocampal dentate gyrus and cerebellum. The selective localization

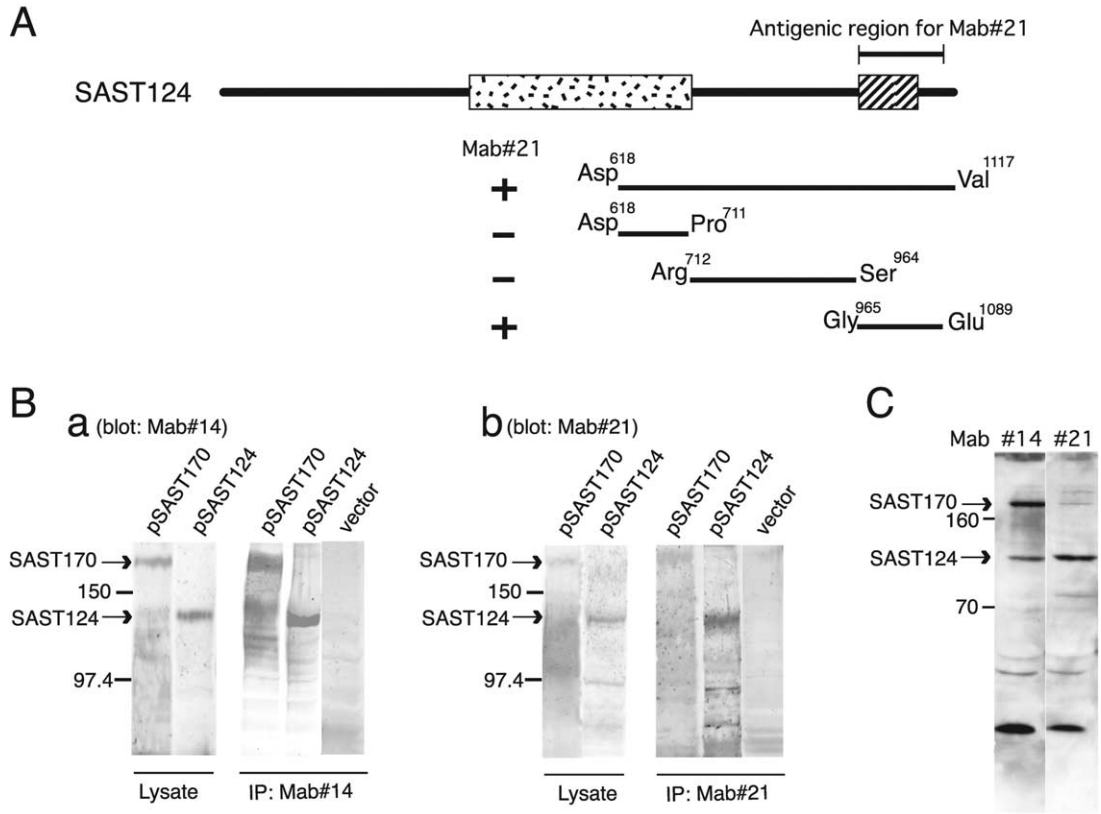

Fig. 3. Specific recognition of SAST124 by mAb#21. (A) The top panel shows the antigenic region for mAb#21 in SAST124 protein. The down panel shows the results of mAb#21 binding to a series of truncated peptides, including Asp^{618}-Val^{1117}, Asp^{618}-Pro^{711}, Arg^{712}-Ser^{964} and Gly^{965}-Glu^{1089} at the C-terminal part of SAST124. (B) Immunoprecipitation and western blotting patterns of HEK293 cell lysate with mAb#14 (a) and mAb#21 (b). The full length cDNA (NruI-*Bam*HI fragment) of SAST124 and SAST170 were cloned into a expression vector (pIRESpuro2, Clontech) for construction of pSAST124 and pSAST170, respectively. Forty-eight hours after transformation with pSAST124, pSAST170 or pIRESpuro2 (vector plasmid), HEK293 cell lysate were prepared and immunoprecipitated by mAb#14 and mAb#21 (Roche et al., 1999). The lysates and precipitates (i.p.) were analyzed by western blotting using 5–20% gradient acrylamide gels (ATTO, Japan). The positions of molecular weight markers (150 kDa and 97.4 kDa) were shown at the left side of the panels. (C) Western blotting patterns of cerebellar lysate with mAb#14 and mAb#21. The lysate (122 μg total proteins in each lane) prepared from adult rat cerebellum (7–14 weeks old male, Wistar/ST), was analyzed by SDS-PAGE using a 7.5% acrylamide gel (Okada, 1996, 1998). Primary antibodies, #14 and #21, were diluted to 0.2 μg/ml and 0.46 μg/ml, respectively, for the reaction. Numbers at the left side of the panel show the position of molecular weight marker proteins (160 kDa and 70 kDa).

of SAST124 in the nuclei of neurons suggested its potential functions in transcriptional regulation in these neurons.

Nuclear localization of SAST124 may be determined by its binding proteins

In the postsynaptic densities receptors and channels are anchored and crosslinked to signaling molecules by PDZ domain proteins to perform synaptic transmission correctly and efficiently (Sheng and Sala, 2001). In most of these cases, the C-terminus of these proteins is protruded to interact with a cavity of the PDZ domain proteins. The C-terminal amino acid sequences of the proteins are classified into three types of consensus sequences (-Ser/Thr-X-Val/Leu, -Φ-X-Φ and –Asp-X-Val) which determine their binding specificity for each PDZ domain. On the other hand, another type of mechanism was proposed for the binding between the PDZ domains of nNOS and α1 syntrophin (Hillier et al., 1999). In this interaction, a region close to the downstream of the PDZ domain in nNOS is taking a β-hairpin structure called β-finger, to form a protrusion for binding to the PDZ domain of α1 syntrophin. The amino acid sequence on the binding face of β-finger contained the consensus amino acid sequence mimicking a peptide ligand for the PDZ domain. The PDZ domain of SAST interacts with the PDZ domain of nNOS or β2 syntrophin, although it lacks the motifs necessary for PDZ domains to interact with peptide ligands (Lumeng et al., 1999). Therefore, SAST PDZ domain may take a β-finger structure to interact with the PDZ domains of nNOS and syntrophin. This idea is supported by the presence of the consensus amino acid sequence (-Ile^{1061}-X-Ile^{1063}-) close to the downstream of the PDZ domain in SAST. If SAST protein is interacting with another partner through its β-finger structure, the difference in amino acid sequence downstream of the PDZ domain between the splice variants may have effects on the binding to their respective partner proteins.

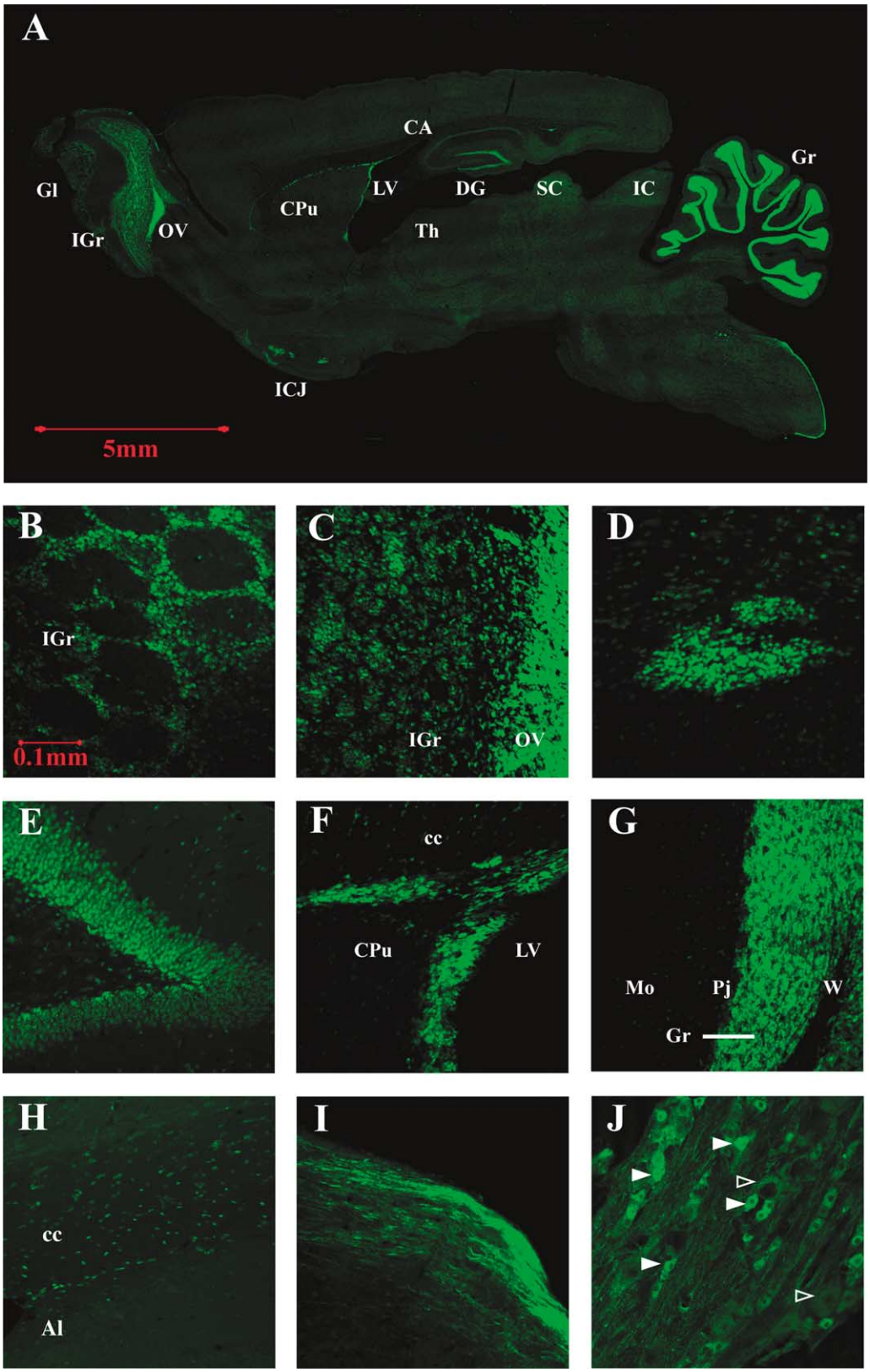
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Fig. 4.

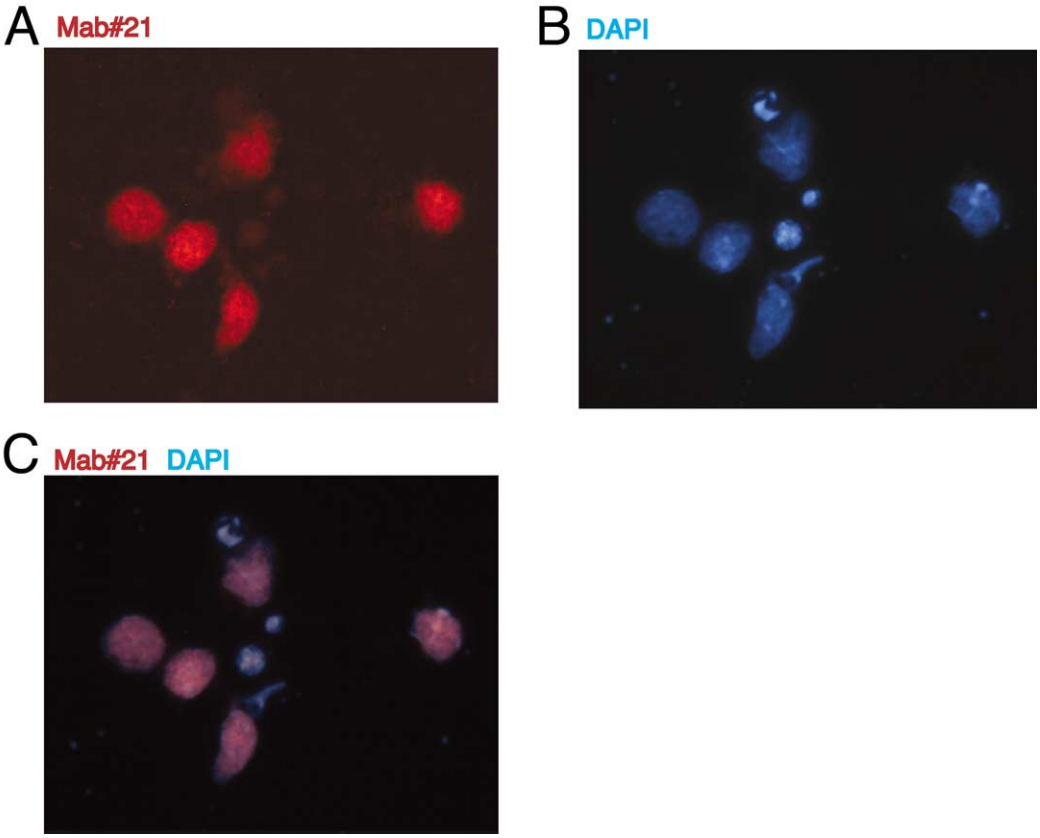

Fig. 5. Selective localization of SAST124 in the nucleus of granule cells. Cerebellar granule cells were prepared from embryonic day 20-21 pups and kept in culture (MEM medium, serum-free, GibcoBRL) for 7 days (Furuya et al., 2000). The culture was used for staining by mAb#21 and DAPI simultaneously. (A) Subcellular distribution of SAST124 observed by mAb#21. Signals were detected with a Cy-3 conjugated secondary antibody (Jackson ImmunoResearch Laboratories, Inc., PA, USA). (B) Nuclear staining by DAPI (2 μg/ml, Sigma, St. Louis, MO, USA). (C) Most of mAb#21 signals are overlapped with DAPI staining.

For localization in the nuclei, proteins require a specific amino acid sequence called a nuclear localization signal (NLS) which interacts with a nuclear transporter complex. SAST124 is selectively localized in the nuclei, however, there is no obvious NLS sequence in SAST124. Therefore, the subcellular localization of SAST124 may be determined by an interaction with its partner protein. The same situation was observed in γ1 syntrophin, which was transported into the nuclei upon interaction with diacylglycerol kinase-ζ (DGK-ζ), otherwise the protein is mainly localized in the perikaryal region and dendrite of neurons in normal state (Hogan et al., 2001). The splicing downstream of the PDZ domain in SAST124 may determine its binding partner to localize it in the nuclei.

SAST170 and SAST124 may share their functions in different subcellular locations

Despite of the difference in the subcellular localization of SAST170 and SAST124, both may share same substrates for their kinase activities, because the N-terminal region including the kinase domain is common in these variants. In some cases, one protein is transported from cell surface region to the nucleus for transduction of signals. For instance, β-catenin is not only stably incorporated into the cadherin-catenin complex to connect extracellular matrix with intracellular cytoskeleton, it is also released from the complex by extracellular signals and transported to the nucleus for regulation of transcription (Wodarz and Nusse, 1998; Dominguez and Green, 2001). Another example is

Fig. 4. Distribution of SAST124 in the brain. (A) The distribution of mAb#21 signals on a parasagittal section of the rat whole brain (4-week-old male, Wistar/ST). Expanded figures of (B) the olfactory bulb; (C) the olfactory ventricular zone; (D) the Islands of Calleja; (E) the hippocampal dentate gyrus; (F) the subventricular zone; (G) the cerebellar cortex; (H) the corpus callosum; (I) the spinal trigeminal and solitary tracts; (J) the trigeminal ganglion. White arrowheads show positively stained small cell bodies, while open arrowheads point negative large cell bodies. Al, alveus hippocampus; CA, hippocampal CA region; cc, corpus callosum; CPu, caudate putamen. DG: hippocampal dentate gyrus. Gl: glomerular layer of the olfactory bulb; Gr, cerebellar granule layer; IC, inferior colliculus; ICJ, Islands of Calleja; IGr, internal granular layer of the olfactory bulb; LV, lateral ventricle; Mo, cerebellar molecular layer; OV, olfactory ventricle; Pj, Purkinje cell layer; s.c., superior colliculus; Th, thalamus; W, cerebellar white matter.

CASK, a member of the membrane-associated guanylate kinase family and functional in assembling molecular components in the synaptic complex, was shown to bind to Tbr-1, a T-box transcription factor, and enter the nucleus for transcriptional regulation (Hsueh et al., 2000). Thus, SAST170 and SAST124 may phosphorylate this type of signaling proteins at the cell surface region and the nucleus, respectively, to regulate their functions in these separated sites.

SAST124 may have specific functions in the restricted brain regions, especially in granule cells

In the adult rat brain, proliferating stem cells are observed in the two regions, the SVZ and the subgranular zone (SGZ) of the hippocampal dentate gyrus (Gage, 2000). After proliferation in the SVZ, neurons tangentially migrate from the zone into the olfactory bulb. Interestingly, SAST124 antibody specifically stained this pathway. These results suggest that SAST124 may be involved in transcriptional regulation in the adult stem cells and their progeny cells in the brain. This hypothesis is supported by the fact that SAST124 is also expressed in granule cells of the hippocampal dentate gyrus, in which stem cells are proliferating to produce granule neurons in the adult. However, no obvious evidence has been observed for existence of the adult stem cells in the Islands of Calleja and cerebellum. The strong expression of SAST124 in these regions is exceptional for the hypothesis. On the other hand, Zipro1 (RU49/Zfp38) is specifically expressed in granule cells of the olfactory bulb, hippocampal dentate gyrus and cerebellum and their progenitor cells throughout life, suggesting their common lineage in the brain (Yang et al., 1996). Hatten and her colleagues also observed that developmental molecular markers for cerebellar granule neurons generally expressed in those of the olfactory bulb and hippocampal dentate gyrus during their development (Alder et al., 1996). From these results, they proposed that these three sets of neuronal precursors share common elements of a developmental program (Hatten et al., 1997; Hatten, 1999). The expression pattern of SAST124 in these neurons is almost completely overlapping that of Zipro1 in the adult brain. Although physiological functions of granule cells are different in each region, these results suggest that some of the common features are conserved among granule cells even in adult, and SAST124 may have regulatory functions for expression of these features in combination with Zipro1.

Apart from the expression in the granule neurons, SAST124 is also localized in glia-like small cells of the corpus callosum and the trigeminal ganglion cells. In these cells, SAST124 is localized in the perikaryal region or axonal process instead of the nucleus. This subcellular localization pattern suggests that SAST124 may have another binding partner for its localization in these cells to perform different functions other than transcriptional regulation.

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