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# A mental retardation gene, motopsin/prss12, modulates cell morphology by interaction with seizure-related gene 6

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ABSTRACT

A serine protease, motopsin (prss12), plays a significant role in cognitive function and the development of the brain, since the loss of motopsin function causes severe mental retardation in humans and enhances social behavior in mice. Motopsin is activity-dependently secreted from neuronal cells, is captured around the synaptic cleft, and cleaves a proteoglycan, agrin. The multi-domain structure of motopsin, consisting of a signal peptide, a proline-rich domain, a kringle domain, three scavenger receptor cysteine-rich domains, and a protease domain at the C-terminal, suggests the interaction with other molecules through these domains. To identify a protein interacting with motopsin, we performed yeast two-hybrid screening and found that seizure-related gene 6 (sez-6), a transmembrane protein on the plasma membrane of neuronal cells, bound to the proline-rich/kringle domain of motopsin. Pull-down and immunoprecipitation analyses indicated the interaction between these proteins. Immunocytochemical and immunohistochemical analyses suggested the co-localization of motopsin and sez-6 at neuronal cells in the developmental mouse brain and at motor neurons in the anterior horn of human spinal cords. Transient expression of motopsin in neuro2a cells increased the number and length of neurites as well as the level of neurite branching. Interestingly, co-expression of sez-6 with motopsin restored the effect of motopsin at the basal level, while sez-6 expression alone showed no effects on cell morphology. Our results suggest that the interaction of motopsin and sez-6 modulates the neuronal cell morphology.


## 1. Introduction

Motopsin (prss12), which is also known as neurotrypsin, is a serine protease secreted by neuronal cells in various brain regions, such as the hippocampus, cerebral cortex, and cranial nerve nuclei [1,2]. This protease is important for neuronal functions because the loss of motopsin function in humans causes severe nonsyndromic mental retardation [3]. Mice lacking motopsin show a decreased number of dendritic spines and reduced phosphorylation of cAMP-responsive element-binding protein (CREB) in the hippocampal neurons, which is induced by social interaction [4]. Furthermore, although axonal injury of facial nerves transiently reduces the expression levels of motopsin mRNA, the recovery of neuronal function accompanies the restoration of the expression of motopsin mRNA, which suggests the involvement of motopsin in neuronal functions [5]. Time-lapse imaging has revealed that depolarization causes the exocytosis of motopsin from presynaptic vesicles [6]. In extracellular space, motopsin cleaves a proteoglycan, agrin [7,8]. As agrin is important for the formation and maintenance of excitatory synapses [9,10], these reports suggest that motopsin may modulate neuronal plasticity via the modification of agrin function.

In a previous report, we indicated that motopsin is detected in the dendrites or in the somatic body of neurons under normal expression levels [2]. The expression of motopsin mRNA in the cerebral cortex culminates around the second week after birth [11,12], and then gradually decreases through the lifetime of an individual. This temporal expression pattern raises the additional possibility that motopsin is involved in the development of the cerebral cortex. The mosaic structure of motopsin, which consists of a signal sequence at the N-terminus followed by a proline-rich domain adjacent to a kringle (proline-rich/kringle) domain, three scavenger receptor cysteine-rich (SRCR) domains, and a protease domain at the C-terminus, suggests that motopsin interacts with other molecules through these domains. Motopsin secreted at

*Abbreviations:* Ade, adenine; ANOVA, analysis of variance; CUB, C1r/C1s, urinary EGF, and bone morphogenetic protein; DIV, days *in vitro*; EGFP-F, farnesylated enhanced green fluorescent protein; IPTG, isopropyl β-D-1-thiogalactopyranoside; P, postnatal day; PBS-T, phosphate-buffered saline containing 0.3% Triton X-100; SD, minimal synthetic dropout medium; SCR, short consensus repeat; SRCR, scavenger receptor cysteine-rich; T-rich, threonine-rich; X-α-gal, 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside.

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synaptic clefts appears to be captured around the synapses [6]. To identify motopsin-interacting proteins, we screened a mouse embryonic cDNA library using a yeast two-hybrid system. We found that the proline-rich/kringle domains of motopsin bound to a transmembrane protein, seizure-related gene 6 (sez-6), which consists of a threonine-rich (T-rich) domain, five short consensus repeat (SCR) domains, two C1r/C1s, urinary EGF, and bone morphogenetic protein (CUB)-like domains, and a transmembrane domain followed by a short cytoplasmic domain at the C-terminal [13]. Biochemical and immunohistochemical analyses indicated that sez-6 is a candidate protein interacting with motopsin in the developing mouse brain.

## 2. Materials and methods

### 2.1. Vector construction

The plasmid vectors used in this study were constructed as described in the Supplemental information and listed below:

pGBK/motopsinS711A: a bait vector expressing motopsinS711A fused with DNA-BD domain.
pGAD/sez-6: a prey vector expressing sez-6 fused with DNA-AD domain.
pET43/proline-rich/kringle: an expression vector to express the NUS-tagged proline-rich/kringle domain of motopsin in *Escherichia coli* (*E. coli*).
pTricHis/SCR/CUB: an expression vector to express the $(His)_6$-tagged SCR/CUB domains of sez-6 in *E. coli*.
pEF1/motopsin: an expression vector to express motopsin without any tag in mammalian cells.
D-HA/motopsin: an expression vector to express HA-tagged motopsin in mammalian cells.
pcDNA/EGFP-F: an expression vector to express farnesylated enhanced green fluorescent protein (EGFP-F) as a control vector.
pcDNA/Sez-6/EGFP-F: an expression vector to express myc-tagged sez-6 and EGFP-F simultaneously in mammalian cells.
pcDNA/motopsin/EGFP-F: an expression vector to express motopsin and EGFP-F simultaneously in mammalian cells.
pcDNA/Sez-6/motopsin/GFP-F: an expression vector to express motopsin, myc-tagged sez-6, and EGFP-F simultaneously in mammalian cells.

### 2.2. Two-hybrid screening

Yeast two-hybrid screening was performed using MATCHMAKER Gal4 Two-Hybrid System 3 (Clontech Lab., Inc., Mountainview, CA) according to the instruction manual. Since protease activity may be toxic to the host cell, we used a mutant lacking protease activity by substituting $Ser^{711}$, which is essential for the proteolytic activity, with Ala (S711A) as a bait protein. Yeast AH109 was transformed by the bait vector pGBK/motopsin S711A, and mated with Y187 strain pretransformed using the mouse E17 cDNA library and plated on minimal synthetic dropout medium lacking adenine, leucine, histidine, and tryptophan (SD/-Ade/-Leu/-His/-Trp). Growing colonies were replated onto SD/-Ade/-Leu/-His/-Trp containing 20 μg/mL 5-bromo-4-chloro-3-indolyl α-D-galactopyranoside (X-α-gal). Complementary DNA from blue colonies was sequenced using T7 primer with an automatic sequencer (ABI 310 sequencer, Applied Biosystems, Foster city, CA).

To clarify the binding domain of motopsin and sez-6, yeast AH109 was transformed by a set of bait and prey vectors, which expressed various kinds of motopsin and sez-6 mutants, respectively, and were assayed on an SD/-Ade/-Leu/-His/-Trp/X-α-gal plate.

### 2.3. Pull-down assay

To prepare a bait protein, an expression vector, pET43/proline-rich/kringle or pET43.1a(+) as a control, was introduced into *E. coli*, Rosetta gami (Merk KGaA, Darmstadt, Germany). The recombinant protein was induced by incubation with 1 mM β-D-1-thiogalactopyranoside in the medium for 3 h. The inclusion body was extracted by sonication in ice-cold lysis buffer (1% Triton X-100, 50 mM Tris–Cl (pH 7.5), 100 mM NaCl) containing protease inhibitors and recovered by centrifugation at $10{,}000 \times g$ at 4 °C for 15 min [15]. The cell lysate was applied to 50 μL of S-protein agarose (Merck KGaA) to bind the proline-rich/kringle domains or NUS-tag protein to the agarose beads.

To prepare SCR/CUB domains of sez-6 fused with an HA-tag, pTricHis/SCR/CUB was introduced into *E. coli*, DH5α. The induction of the recombinant protein and cell lysis were performed as above. Inclusion bodies were recovered by centrifugation and dissolved in 20 mM Tris–Cl (pH 7.5), 150 mM NaCl, and 8 M urea, and then applied to the S-protein agarose possessing NUS-tagged proline-rich/kringle domains or only NUS tag. The agarose was washed with 20 bed volumes of 20 mM Tris–Cl (pH 7.5), 150 mM NaCl, and 0.1% Triton X-100. The bound proteins were eluted in 60 μL of SDS-sample buffer (50 mM Tris–Cl, pH 6.8, 2% SDS, 10% glycerol, 50 mM 2-mercaptoethanol, 0.1% bromophenol blue) and detected by Western blotting using anti-HA tag antibody (Life Technologies Corp., Carlsbad, CA).

### 2.4. Cell cultures

The preparation and culture of primary hippocampal neurons were performed as described elsewhere [2,14]. The cells were cultured in serum-free Neurobasal medium containing B27 supplement (Life Technologies Co.) at a density of $3 \times 10^4$ cells/cm$^2$ on a glass slide with four glass-surface wells in water-repellant print (Matsunami Glass Ind., Ltd., Osaka, Japan), which was coated with poly-D-lysine and laminin. At 1 day *in vitro* (DIV) or 3 DIV, the cells were transfected with 0.3 μg of an expression plasmid for mammalian cells by magnetofection using a NeuroMag kit (OZ Biosciences, Marseille cedex, France) according to the manufacturer's manual.

The mouse neuroblastoma cell line Neuro-2a (ATCC No. CCL-131) and COS1 (ATCC No. CRL-1650) cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies Co.) containing 7% fetal bovine serum at 37 °C in 5% $CO_2$.

### 2.5. Immunoprecipitation

COS1 cells were plated on a six-well plate and transfected with 1 μg of D-HA/motopsin and 2 μg of pcDNA3.1/mouse Sez-6-Myc using Fugene 6 (Roche Applied Sciences, Mannheim, Germany) according to the instruction manual. After 2 days, the cells were lysed in 0.5 mL of ice-cold RIPA buffer (20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate). The lysate was centrifuged at $10{,}000 \times g$ for 10 min at 4 °C. The supernatant was incubated with 2 μg of rabbit IgG for 2 h at 4 °C, and then incubated with 10 μL of Protein A Sepharose (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) for 3 h. The lysate was then centrifuged at $5000 \times g$ for 5 min at 4 °C. The supernatant was supplemented with 2 μg of rabbit anti-Myc tag IgG (Sigma–Aldrich Co., St. Louis, MO) and incubated at 4 °C overnight. Protein A Sepharose was added to the reaction mixture and incubated for 3 h. Protein A Sepharose was then recovered after centrifugation at $5000 \times g$ for 5 min at 4 °C, and washed with 1 mL of washing buffer (20 mM Tris–Cl, pH 8.0, 136 mM NaCl, 0.05% Tween-20) three times. The bound proteins were eluted in 40 μL of SDS-sample buffer.

### 2.6. Immunohistochemistry

Immunohistochemistry was performed as previously described [14]. In brief, mice were perfused transcardially with ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) on postnatal day (P) 7. Brains were postfixed at 4 °C for 18 h and then transferred into 20% sucrose in 0.1 M phosphate buffer (pH7.4). Specimens were sectioned at a thickness of 22 μm using a cryostat. The sections were reacted with anti-sez-6 antibody labeled with Alexa 594 and anti-motopsin antibody labeled with Alexa 488 (dilution, 1:1000) in phosphate-buffered saline containing 0.3% Triton X-100 (PBS-T) overnight at 4 °C. The labeling of antibodies was carried out using a Zeon Antibody Labeling Kit (Life Technologies Corp.). The specimens were mounted on slide glasses after washing with PBS-T. Fluorescent images were taken using a confocal microscope (FV-100D, Olympus, Tokyo, Japan).

Human brain specimens were purchased from the Biochain Institute (Hayward, CA). Immunofluorescence hardly detected either motopsin or sez-6 in the human tissue. Immunohistochemistry using avidin–biotin complex was performed. After antigen retrieval in 10 mM sodium citrate buffer containing 0.05% Tween 20, specimens were stained with anti-motopsin or anti-sez-6 antibody (1:1000). The specimens were reacted with biotinylated anti-rabbit IgG antibody (1:1000), and then biotin–avidin complex of peroxidase using a Vectastain ABC kit (Vector Laboratory, Burlingame, CA). Images were taken using a light microscope (Eclipse 80i, Nikon, Tokyo, Japan) and a cooled CCD camera (VB-7000, Keyence Corp., Osaka, Japan).

### 2.7. Morphological analysis

Neuro2a cells ($3 \times 10^4$) were inoculated on a glass slide with four glass-surface wells in water-repellant print one day before transfection. Cells were transfected with 0.3 μg of a plasmid (pcDNA/EGFP-F, pcDNA/motopsin/EGFP-F, pcDNA/Sez-6/EGFP-F, or pcDNA/Sez-6/motopsin/EGFP-F) using FuGENE 6. Two days after

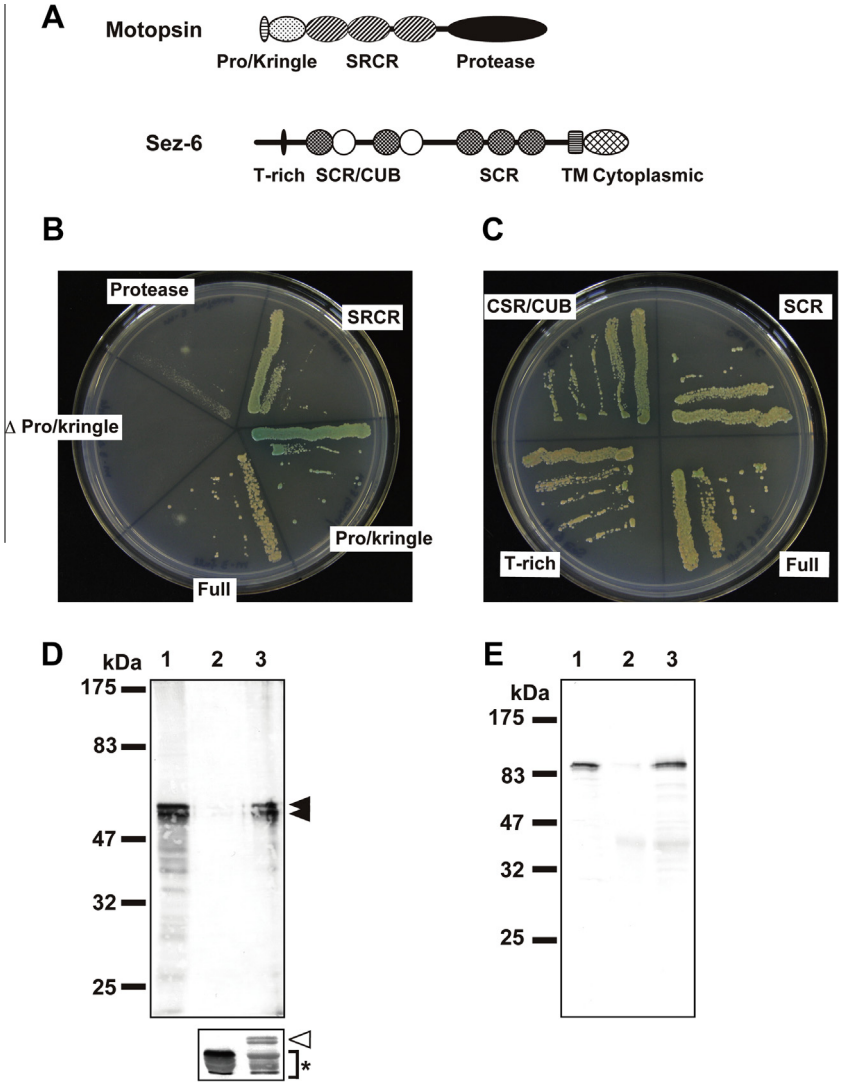


**Fig. 1.** The interaction between motopsin and sez-6. (A) Schematic structures of motopsin and sez-6 are shown. (B) Motopsin interacted with sez-6 through a proline-rich/kringle domain. Yeast cells expressing sez-6-AD fusion protein and the indicated motopsin mutant fused with DNA-BD were plated on SD/-Ade/-Leu/-His/-Trp/X-α-gal. (C) Yeast cells expressing motopsin-DNA-BD fusion protein and the indicated sez-6 mutant fused with AD were grown as above. (D) Pull-down assay suggested that the proline-rich/kringle domain interacted with sez-6. The CUB/SCR domain of sez-6 (black arrowheads) bound to the agarose possessing the proline-rich/kringle domain (a white arrowhead), but not NUS protein (an asterisk). Lane 1, total lysate; lane 2, eluted protein from NUS-tag-fixed agarose; lane 3, eluted protein from agarose possessing the proline-rich/kringle domains. (E) Immunoprecipitation assay suggested the interaction of motopsin and sez-6. The cell lysates were immunoprecipitated using anti-myc antibody, and the precipitated protein was detected using anti-HA tag antibody. Lane 1, total lysate; lane 2, immunoprecipitate from cells expressing only HA-tagged motopsin; lane 3, immunoprecipitate from cells expressing both HA-tagged motopsin and myc-tagged sez-6.

transfection, the cells were immunocytochemically stained according to a previous report [14]. The first antibodies used in this experiment and the dilution rate were as follows: 1:1000 rat anti-GFP IgG, 1:1000 rabbit anti-motopsin IgG, and 1:500 mouse anti-myc IgG (clone MC045, Nacalai Tesque Inc., Kyoto, Japan). Then, the cells were reacted for 2 h at room temperature with secondary antibodies at 1:1000 dilution. The used secondary antibodies were anti-rat IgG/Alexa 488, anti-rabbit IgG/Alexa 405, and anti-mouse IgG/Alexa 594 (LifeTechnologies Corp.). The slide was mounted with Prolong Gold Antifade Reagent (Life Technologies Corp.) and observed using a confocal microscope (FV-100D, Olympus).

Only cells confirmed to express the transfected cDNA were analyzed. The observation of neurite outgrowth was performed according to Bryan et al. [16]. A neurite was defined as an outgrowth with a length of more than half the diameter of the cell body. The length of neurites and the diameter of cell bodies were measured using ImageJ software (version 1.43, NIH). The numbers of neurites and filopodia-like structures on neurites as well as the number of branches of neurites were manually counted. The data were analyzed using JMP software (SAS Institute Inc., Cary, NC). Statistical comparisons were performed using analysis of variance (ANOVA) and Tukey–HSD post hoc tests for pairwise comparisons. $p$ values <0.05 were considered significant.

## 3. Results

We screened $5 \times 10^7$ colonies of a mouse E17 cDNA library with inactive motopsin mutant (S711A) as bait using a yeast two-hybrid system. Sequence analyses of 96 positive clones revealed that several cDNAs encoded proteins located in extracellular space or on the plasma membrane. Among them, we further analyzed a clone encoding sez-6 (Fig. 1A) for the following reasons. Sez-6 is a transmembrane protein located on the plasma membrane of neuronal cells [19]. It is expressed in hippocampal and cortical neurons, which also express motopsin [14]. Furthermore, sez-6 is known to modulate synaptogenesis and dendritic arborization of cortical neurons [17].

First, we identified the binding region of each protein, since motopsin is a multidomain protease (Fig. 1A). Yeast transformed with a cDNA encoding the proline-rich/kringle domain or SRCR domains of motopsin made colonies on SD/-Ade/-Leu/-His/-Trp/X-α-gal plates, whereas yeast having the cDNA encoding the protease domain did not survive on the same medium (Fig. 1B). The colonies expressing the proline-rich/kringle domains were bluer than those expressing SRCR domains, suggesting that the proline-rich/kringle domain bound to sez-6 more strongly than the SRCR domains did. Yeast transformed with a mutant lacking the proline-rich/kringle domain (Δproline-rich/kringle in Fig. 1B) did not grow on an SD/-Ade/-Leu/-His/-Trp/X-α-gal plate, suggesting the significant contribution of this domain to the binding to sez-6. Similarly, deletion mutants of sez-6 cDNA were also investigated to identify the domain of binding to motopsin. Whereas yeast expressing SCR/CUB domains showed the bluest color, yeast transformed with any mutant grew on SD/-Ade/-Leu/-His/-Trp/X-α-gal plates, suggesting that all domains of sez-6 had the ability to bind to motopsin (Fig. 1C).

To confirm the interaction between motopsin and sez-6, we performed a pull-down assay. *E. coli* lysate containing SCR/CUB

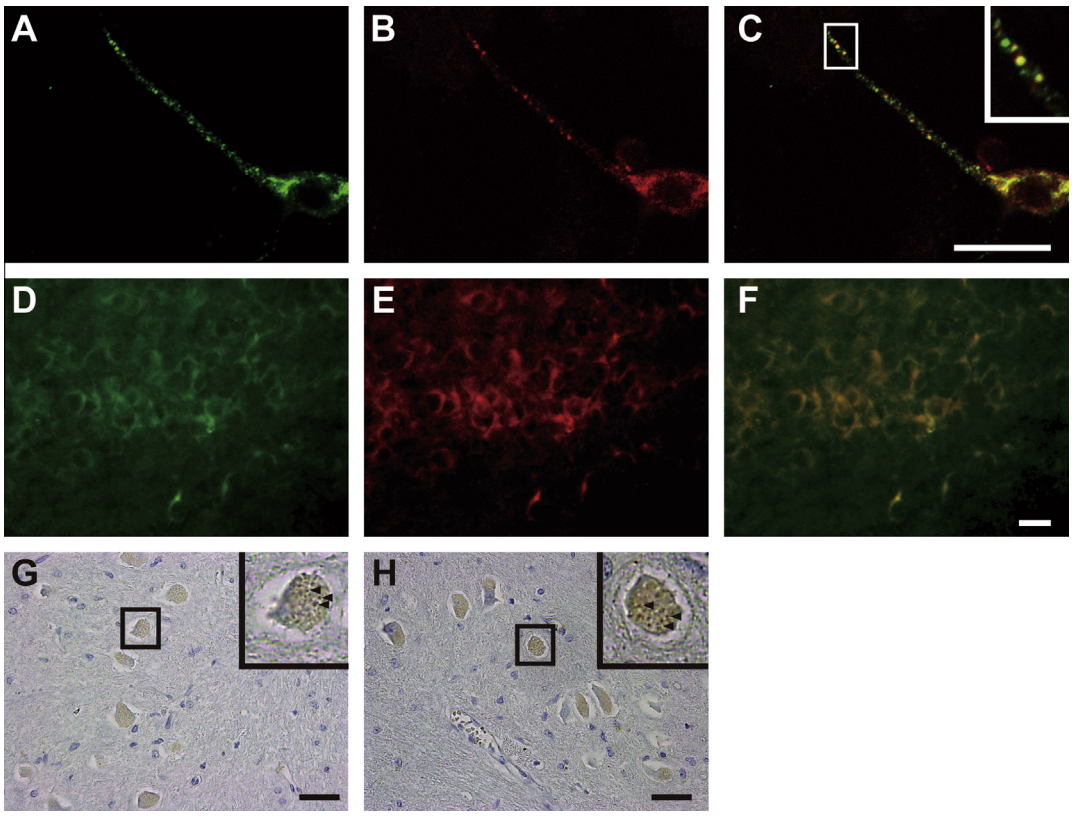


**Fig. 2.** Co-localization of motopsin and sez-6 in neuronal cells. (A) Motopsin was detected in discrete puncta in cultured hippocampal neurons. (B) The localization of sez-6 was similar to that of motopsin in neuronal cells. (C) A merged image clearly shows the co-localization of these proteins in intracellular vesicles. (D) Motopsin was detected in cortical neurons of P7 mouse brain. (E) Sez-6 protein was also stained. (F) A merged image indicates that both proteins were co-localized in cortical neurons of P7 mouse brain. (G) Motopsin was detected in motor neurons in the human spinal cord. A magnified image of the boxed region shows the punctate pattern of motopsin localization in a somatic body (arrowheads). (H) Sez-6 was detected in motor neurons in the anterior horn. A magnified image shows that the localization of sez-6 in a soma was quite similar to that of motopsin (arrowheads). Bars, 20 μm.

domains fused with an HA tag was applied to the S-protein agarose possessing a proline-rich/kringle domain or NUS tag. Western blot analysis using anti-HA antibody showed that the SCR/CUB domain of sez-6 bound to only S-protein agarose possessing the proline-rich/kringle domain, but not the NUS-tag-fixed agarose (Fig. 1D). Furthermore, HA-tagged motopsin and myc-tagged sez-6 expressed in COS cells were co-immunoprecipitated. The immunoprecipitation by an anti-myc antibody recovered HA-tagged motopsin from the lysate of cells expressing both proteins. However, the expression of motopsin alone did not lead to the co-precipitation of motopsin by the anti-myc antibody (Fig. 1E). Immunocytochemistry using an anti-myc antibody showed a

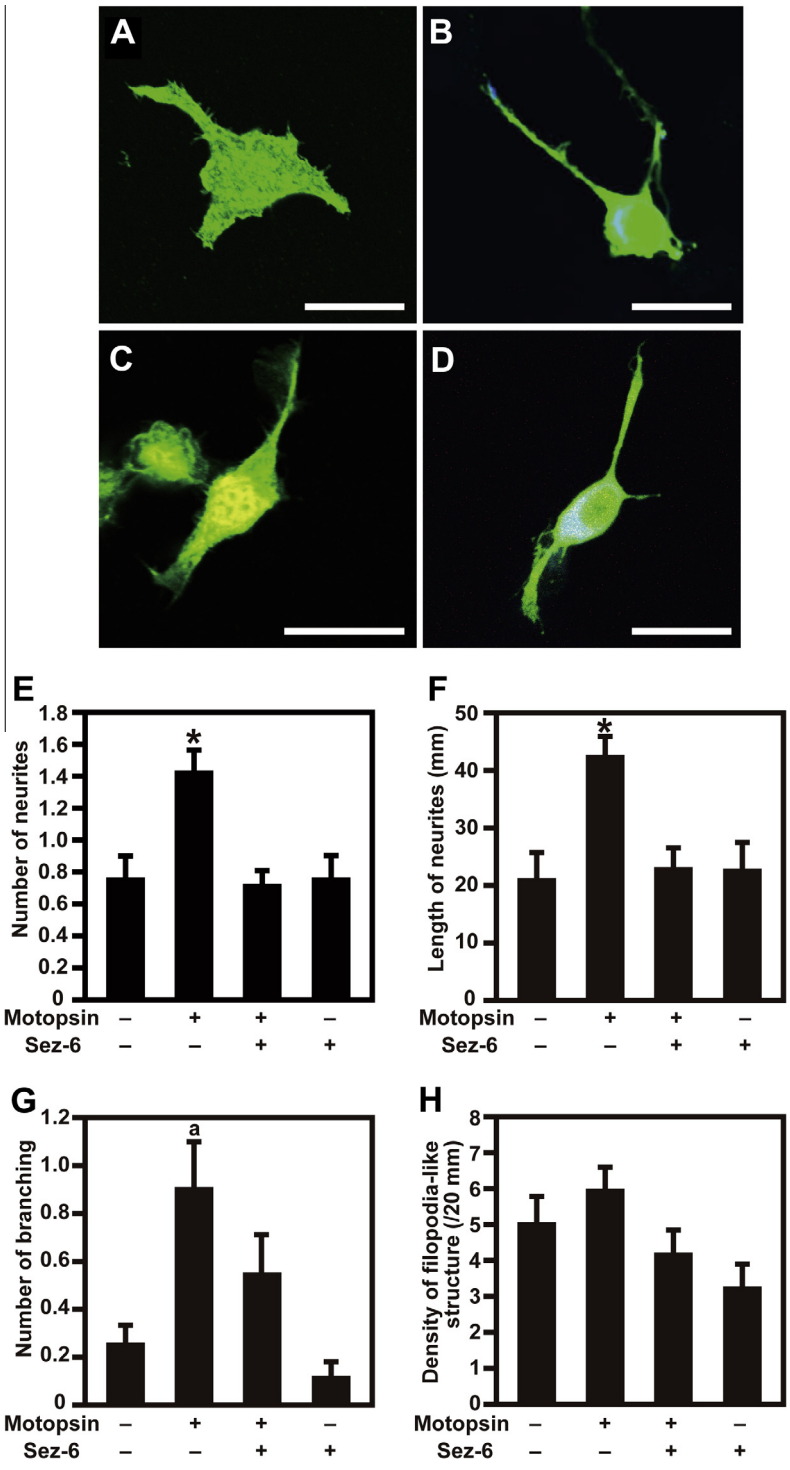


**Fig. 3.** Effects of co-expression of motopsin and sez-6 on cell morphology. Typical cell morphology of neuro2a cells expressing only EGFP-F (A), motopsin (blue, B), sez-6 (yellow, C), or both motopsin and sez-6 (white, D) is shown. Bars, 20 μm. (E) Motopsin expression significantly increased the number of neurites ($^{*}p < 0.05$). However, co-expression of sez-6 with motopsin decreased it to the basal level. (F) The length of neurites of cells expressing only motopsin was significantly greater than that of other cells ($^{*}p < 0.05$). (G) The expression of only motopsin significantly increased the number of branches ($^{a}p < 0.05$). Co-expression of sez-6 with motopsin appeared to restore this, albeit not significantly. (H) The expression of motopsin and/or sez-6 showed no effects on the density of filopodia-like structures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

punctate pattern in the neurites of neuronal cells that expressed myc-tagged sez-6 (Fig. 2B). This distribution pattern was similar to that of motopsin. The merged image clearly showed that motopsin and sez-6 co-localized within the speckles (Fig. 2C, enlarged image). The results of the co-immunoprecipitation and the immunocytochemical analyses in cultured neurons suggest the ability of motopsin to bind sez-6 under physiological conditions.

To confirm the co-localization of motopsin and sez-6 in the neural tissues, fluorescent immunohistochemistry was performed. Both motopsin and sez-6 were detected on somatic bodies of the cortical neurons at P7. The merged image showed the co-localization of these proteins in the neuronal cells (Fig. 2F). Although motopsin was detected in cortical neurons in the adult human brain, sez-6 was undetectable (data not shown). In the human spinal cord, immunohistochemistry showed similar localization of motopsin and sez-6. Both proteins were detected as speckles in neuronal cells at the anterior horn (insets in Fig. 2G and H).

To understand the biological function of the interaction between motopsin and sez-6, a combination of these proteins or each protein individually with EGFP-F was transiently expressed in neuro2a cells. When only sez-6 was expressed, the cell morphology was similar to moc-transfected cells expressing only EGFP-F. The expression of motopsin significantly increased the number of neurites, and elongated their length (Fig. 3, post hoc analysis after one-way ANOVA: neurite number, $F(3, 213) = 17.81$, $p < 0.0001$; neurite length, $F(3, 210) = 22.15$, $p < 0.0001$). The expression of motopsin increased the number of branches compared with that of moc-transfected cells (post hoc analysis after one-way ANOVA: $F(3, 223) = 5.49$, $p = 0.0012$). Interestingly, the co-expression of sez-6 with motopsin opposed the effects of motopsin. Both the number and the length of neurites declined to the basal levels when motopsin and sez-6 were co-expressed (Fig. 3E and F). The co-expression of sez-6 and motopsin appeared to restore the increased number of branches, but this was not significant (Fig. 3G). None of the expression of both motopsin and sez-6 or each protein individually affected the density of filopodia-like structures on neurites (Fig. 3H).

## 4. Discussion

Motopsin is secreted from neuronal cells in an activity-dependent manner and captured around synapses [6], although the binding partner is still obscure. We identified sez-6 as a motopsin-binding protein using a yeast two-hybrid system. The analyses of deletion mutants of motopsin revealed that the proline-rich/kringle domains are critical for the interaction between motopsin and sez-6. The binding domain and protease domain are located at the N-terminus and C-terminus, respectively. Motopsin bound to sez-6 appears to cleave substrates around neuronal cells, since sez-6 is a transmembrane protein localized on the plasma membrane of neuronal cells. Recent reports indicate that motopsin releases a 22-kDa C-terminal fragment from a proteoglycan, agrin, and the fragment reduces $Na^+/K^+$ ATPase on postsynaptic spines, which results in increased action potential firing [8,18]. Motopsin trapped around the cell membrane may promote the binding of agrin C-terminal fragment to $Na^+/K^+$ ATPase.

In addition to the interaction between these proteins in the yeast system, biochemical analyses (Fig. 1D and E) and analysis of the co-localization in neuronal cells (Fig. 2) strongly suggest the interaction of motopsin and sez-6. Both protein levels are relatively high in the cerebral cortex and hippocampus in mouse brain at around 10 days old [2,11,14]. Like the secretion of motopsin, the expression of sez-6 is up-regulated by neuronal activity [19]. Pyramidal neurons in the cortex of sez-6-deficient mice exhibit an excess of short dendrites [17]. Furthermore, sez-6 is known to regulate synaptogenesis and neuronal plasticity, since sez-6-deficient neurons show a decreased density of excitatory synapses on cortical neurons and diminished excitatory post-synaptic responses. Some of this phenotype of sez-6 deficiency is similar to that of motopsin deficiency. Motopsin deficiency also causes decreased density of excitatory synapses in the hippocampus and diminishes LTP-associated formation of filopodia [4,20]. The similarity of the phenotypes shown by motopsin- and sez-6-deficient mice, in addition to the overlapping distribution of motopsin and sez-6, supports the interaction between these proteins in mouse brain.

Overexpression of motopsin enhanced the number and length of neurites, as well as the number of branches (Fig. 3). This result is consistent with a previous report showing that the overexpression of motopsin in motor neurons results in excessive axonal elongation [21]. Interestingly, the co-expression of sez-6 with motopsin completely restored the effects of motopsin on neurite elongation, while the expression of only sez-6 showed no significant effect. These results suggest that the interaction with sez-6 modulates motopsin function, at least in that it reinstates neurite elongation and branching induced by motopsin expression in neuro2a cells. Further investigations are apparently necessary to understand the biological functions of the interaction between motopsin and sez-6 in the developing mammalian brain.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.04.112.

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