BH-protocadherin-c, a member of the cadherin superfamily, interacts with protein phosphatase 1 alpha through its intracellular domain

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Abstract Using a yeast two-hybrid system, we isolated eight cDNA clones which interacted with BH-protocadherin-c (BH-Pcdh-c) from the human brain cDNA library. One clone encoded protein phosphatase type 1 isoform α (PP1α) and another two PP1α2. PP1α was co-immunoprecipitated from the extract of a gastric adenocarcinoma cell line MKN-28 with anti-BH-Pcdh-c antibody. PP1α activity towards glycogen phosphorylase was inhibited by the intracellular domain of BH-Pcdh-c. Inhibition of the phosphatase required more than the minimal domain of BH-Pcdh-c which could associate with PP1α. In situ hybridization revealed that BH-Pcdh-c mRNA was predominantly expressed in cerebral cortex neurons in the adult mouse brain.

Key words: BH-protocadherin-c; Two-hybrid system; Protein phosphatase type 1; Cerebral cortex

1. Introduction

The cadherin superfamily can be classified into two groups, classic type and non-classic type [1,2]. Although the intracellular domain of classic cadherins is well known to associate with catenins [3], there is little information about the molecules, which interact with that of protocadherins (Pcdhs), the major non-classic type subfamily [4]. The intracellular domain of Pcdhs lack the homologous sequence corresponding to the βcatenin-binding site described for the classic cadherins [5]. Immunoprecipitation and affinity chromatography experiments showed there is no obvious interaction between Pcdhs and catenins [4,6]. It is possible that the intracellular domain of Pcdhs associates with proteins other than catenins. Indeed, cadherin-related neural receptor, a novel non-classic type subfamily, was recently identified to interact with Fyn [7].

Pcdhs were originally isolated from the vertebrate central nervous system [4]. Many Pcdhs appear to be expressed predominantly in brain and individual Pcdhs have a characteristic expression pattern in brain [8–12]. Although Pcdhs seem to be involved in some highly specialized cell-cell interaction processes in the central nervous system [4], their biological functions remain to be identified.

Previously, we isolated human BH-protocadherin (BH-Pcdh) which showed overall significant homology with Pcdh1 [13]. BH-Pcdh also exhibited 85% identity with *Xenopus* NF-protocadherin, which was shown to be required for *Xenopus* ectodermal differentiation [6]. There are three isoforms of BH-Pcdh, denoted -a, -b and -c, which have a different cytoplasmic tail. Interestingly, the intracellular domain of BH-Pcdh-c showed similar length and significant homology with that of an alternatively spliced product of Pcdh1 [13]. We consider that this well-conserved C-terminus region between BH-Pcdh-c and Pcdh1 isoform suggests the functional importance of this region.

To identify novel cellular proteins associated with the intracellular domain of BH-Pcdh-c, we performed yeast two-hybrid screening using human brain cDNA library. Here we report the in vitro and in vivo interaction between BH-Pcdh-c and protein phosphatase type 1 isoform α (PP1α). Furthermore, we performed in situ hybridization using adult mouse brain and observed the localization of BH-Pcdh-c mRNA predominant in the cerebral cortex neurons.

2. Materials and methods

2.1. Yeast two-hybrid system

Two-hybrid screening [14] was performed using the Matchmaker two-hybrid system from Clontech. The intracellular domain of BH-Pcdh-c (GenBank Accession Number. AB006757; amino acids 848–1200) was cloned by PCR with primers 5′-CGCCATGGTAGT-GATGGCAAGGTACTGCAGGTCC-3′ and 5′-CGGTCGACGCT-GTTGCTCCTTTATAGCCTACAGG-3′. The resulting PCR product was cleaved with *Nco*I and *Sal*I and cloned into the corresponding site of GAL4 DNA binding domain expression vector pAS2 (Clontech) as a bait. cDNAs derived from human brain mRNA were directionally subcloned into the *Eco*RI/*Xho*I digested GAL4 activation domain expression vector pACT2 (Clontech) as a prey. *Saccharomyces cerevisiae* HF7c was transformed by the lithium acetate method [15] and positive colonies were screened for β-galactosidase activity using a filter lift assay.

2.2. Stable transfection of human BH-Pcdh-c in L cells

The full-length human BH-Pcdh-c cDNA was digested with *Fse*I and *Hin*dIII in order to remove extra ATG sequences located in the 5′-untranslated region. The 3.8-kb of the fragment including the complete coding sequence was subcloned into the expression vector driven by the human elongation factor 1α promoter and the human cytomegalovirus enhancer [16]. For a control, the expression vector without cDNA insert was also used. LTK$^-$ cells (Riken Cell Bank; No. RCB0208) were transfected with Lipofectamine (Life Technologies). After 48 h, the transfected cells were selected with 600 μg/ml G418 (Wako). Northern blot analysis showed the expression of mRNA of the expected sizes in the transfected cells and the clones exhibiting high expression of BH-Pcdh-c were used for the further analysis. Isolated clones were maintained in MEM medium supplemented with 10% bovine calf serum at 37°C in a 5% CO_2 atmosphere.

2.3. Antibody production

Anti-serum was raised in rabbits using a peptide corresponding to a sequence in the C-terminus of BH-Pcdh-c (EYKPSPVNTLTRREV-YL) conjugated to keyhole limpet hemocyanin. BH-Pcdh-c antibody specificity was checked by Western blotting using BH-Pcdh-c trans-

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fected L cells. As control, parent L cells or MOCK was used. In the transfected cells, the expected 160-kDa protein was detected (Fig. 2C).

2.4. Cell extract preparation

Human gastric adenocarcinoma cells MKN28 were grown in RPMI 1640 supplemented with 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere. Confluent cells were washed twice with cold phosphate-buffered saline, pH 7.4 (PBS) and scraped on ice in PBS. Collected cells were incubated in the lysis buffer (1% Triton X-100 for immunoprecipitation or 1% Nonidet P-40 for pull-down assay, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 g/ml leupeptin, 10 g/ml aprotinin) with constant shaking for 30 min at 4°C and spun at 12 000 rpm for 30 min. The supernatant was used as soluble fraction for further experiments.

2.5. Preparation of GST fusion proteins and pull-down assay

To construct GST fusion proteins, amino acids corresponding to 877–1200 (GST-CP), 1009–1200 (GST-RVTF (+)) and 1020–1200 (GST-RVTF (−)) of BH-Pcdh-c were cloned by PCR using the following sense primers: for GST-CP, 5′-CGGAATTCTACACCCCAACAGCATGACAAATCTA-3′; for GST-RVTF (+), 5′-CGGAATTCCAGCAAACAGCCATTTCGTAGAGTGA-3′; for GST-RVTF (−), 5′-CGGAATTCTGTTGTGAGTCAGCCTCAGGACCCAC-3′ and the common antisense primer: 5′-CGGAATTCGCTGTTGCTCCTTTATAGCCTACAGG-3′. The resulting PCR products were cleaved with *Eco*RI and cloned into the corresponding site of pGEX-3X (Pharmacia). The protein was expressed in *E. coli* DH5α by induction of log phase cells with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside at 37°C for 4 h. Cells were resuspended in ice-cold TNE buffer containing 50 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM EDTA and 1 mM dithiothreitol. Following sonication using Branson sonifier 250, Triton X-100 was added to 1%. The supernatant was directly added to glutathione-Sepharose 4B (Pharmacia) and washed extensively with PBS. The fusion protein was eluted with 16 mM reduced glutathione in TNE buffer. For pull-down experiments, similar quantities of purified GST or GST fusion proteins were bound to glutathione-Sepharose 4B beads in binding buffer containing 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 100 mM NaCl, 10% glycerol, 0.5 mg/ml bovine serum albumin and 5 mM 2-mercaptoethanol for 1 h at 4°C. Followed by washing, the GST fusion protein bound to beads was incubated with extracts made from MKN28 cells for 1 h at 4°C, then washed four times with binding buffer to remove unbound proteins. Subsequently, the specifically bound proteins were eluted with a solution containing 10 mM reduced glutathione in binding buffer without bovine serum albumin and analyzed by Western blotting with anti-PP1α polyclonal antibody (Upstate Biotechnology; dilution 1:1000) and anti-PP1α monoclonal antibody (Transduction Laboratories; dilution 1:1000), respectively. Labeled proteins were detected by Western blot solution obtained from Promega.

2.6. Immunoprecipitation

The lysates of MKN28 cells or L cells expressing BH-Pcdh-c were precleared with protein G-Sepharose (Pharmacia) equilibrated in PBS containing 0.1% bovine serum albumin and 0.05% Tween 20, for 30 min at 4°C under shaking. After a short spin, the supernatant was transferred into a new tube and incubated under shaking with the respective antibody for 1 h at 4°C. The immunocomplexes were absorbed to protein G-Sepharose for 1 h at 4°C and washed four times in the lysis buffer. Associated proteins were then eluted in 2×SDS-PAGE loading buffer by boiling for 5 min and analyzed by Western blotting.

2.7. Protein phosphatase assay

^{32}P-labelled glycogen phosphorylase (Life Technologies) and myelin basic protein (New England Biolabs) were prepared according to the manufacturer's instruction. The specific activity of the [γ-^{32}P]ATP used for phosphorylation was 10^6 cpm/nmol. PP1α was purchased from New England Biolabs. The phosphatase assay (final volume 60 μl) was carried out with 0.05 μg/ml of PP1α in 15 mM Tris-HCl, pH 7.0, 13 mM imidizol-HCl, pH 7.63, 3% β-mercaptoethanol, 0.3 mg/ml of bovine serum albumin, 5 mM caffeine, 0.1 mM EDTA, 1 mM $MnCl_2$ with or without varied concentration of GST, GST-CP, GST-RVTF(+) and GST-RVTF(−). The reaction was initiated by addition of 1 mg/ml ^{32}P-labelled substrate, carried out for 10 min at 30°C and stopped by trichloroacetic acid to a final concentration of 15%. Cherenkov counting determined radioactivity present in the supernatant.

2.8. Animals and section preparation

Male mice (C57BL/6J), at 10 weeks of age, were killed with pentobarbital. The brain was removed and fixed with 4% paraformaldehyde (PFA)/PBS for 24 h followed by 30% sucrose/PBS for 24 h. The thin sections (20 μm) were air dried on gelatin-coated glass slides and subjected to 4% PFA/PBS fixation for 15 min at room temperature.

2.9. In situ hybridization

Antisense and sense digoxigenin (DIG)-labeled cRNA probes were synthesized using the DIG RNA labeling kit (Boehringer Mannheim). As template, the 0.6-kb coding region of BH-Pcdh-c (nucleotides 3879–4477) subcloned in the *Eco*RI site of pBluescript SK(−) was used. DIG-labeled cRNA was detected using the DIG nucleic acid detection kit (Boehringer Mannheim). Briefly, each section was incubated with alkaline phosphatase-conjugated anti-DIG antibody (dilution 1:3000) at room temperature for 1 h and phosphatase activity visualized with NBT/BCIP (nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate). The sections were then counter-stained with methyl green.

3. Results

3.1. Identification of a novel cellular protein interacting with the intracellular domain of BH-Pcdh-c

To identify cellular proteins capable of interacting with the

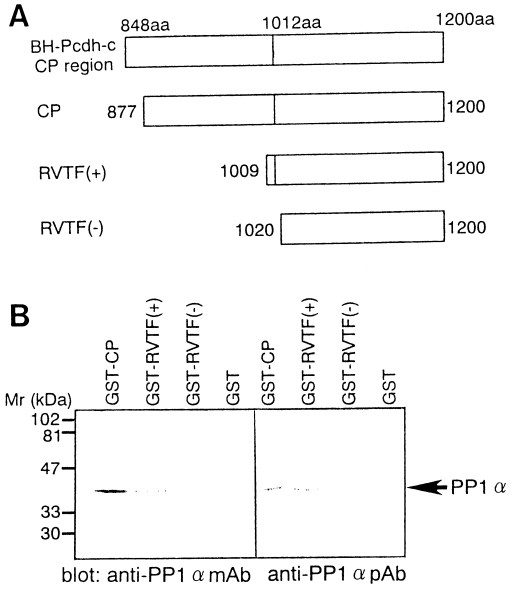

Fig. 1. In vitro association of PP1α with the intracellular domain of BH-Pcdh-c. A: Schematic diagram of the intracellular domain of BH-Pcdh-c used in the pull-down assay. BH-Pcdh-c fragments containing almost the full-length cytoplasmic region (CP) and the C-terminus half region with (+) or without (−) RVTF sequence are aligned with the full length BH-Pcdh-c intracellular domain. Numbers indicate amino acid positions with respect to the original BH-Pcdh-c sequence. Positions of the splice site (1012th amino acid) are indicated. B: To perform pull-down assay, amino acid 877–1200 (GST-CP), 1009–1200 (GST-RVTF(+)) and 1020–1200 (GST-RVTF (−)) of BH-Pcdh-c were fused to GST. GST fusion proteins were bound to glutathione-Sepharose beads in binding buffer and incubated with MKN28 cell lysates. Following extensive washing, complexes eluted from beads were subjected to Western blotting with rabbit polyclonal (pAb) and mouse monoclonal (mAb) anti-PP1α antibody. GST alone was used as a negative control. The specific 37-kDa band is indicated by the arrow on the right. The relative molecular weights of prestained molecular weight markers are noted on the left.

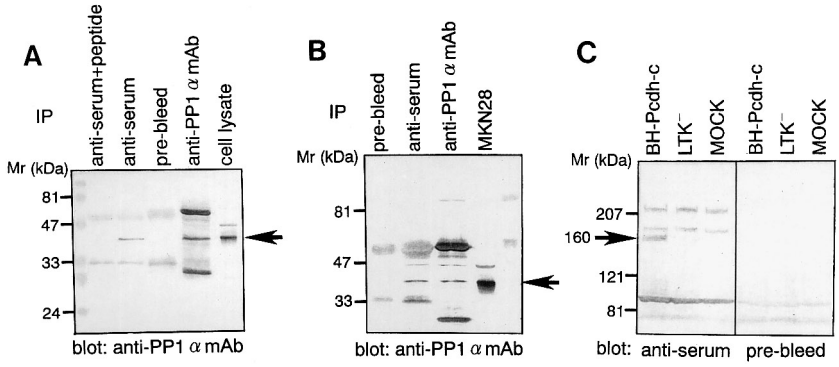

Fig. 2. In vivo association of PP1α with BH-Pcdh-c. L cells expressing BH-Pcdh-c (A) and MKN28 cells (B) were lysed and incubated with the indicated antibody (upper side) and protein G-Sepharose. Following extensive washing, complexes were assayed for PP1α content by Western blotting with an anti-PP1α monoclonal antibody (mAb). To confirm the specificity of immunoprecipitation for anti-BH-Pcdh-c antibody, synthetic peptide corresponding to the C-terminus of BH-Pcdh-c was added to the anti-BH-Pcdh-c antibody before immunoprecipitation (lane 1 of A). The cell lysates and anti-PP1αmAb immunoprecipitates were used for confirming the position of PP1α. The specific 37-kDa band is indicated by the arrow on the right. C: Immunoblot analysis of BH-Pcdh-c transfected L (LTK^-) cells. The cell extracts were subjected to Western blotting. Anti-BH-Pcdh-c antibody stained a 160-kDa band only in BH-Pcdh-c transfectant, but not in parent or MOCK transfected cells (left panel). Pre-bleed detected no bands in any cells (right panel). The 160-kDa band is indicated by the arrow on the left. The positions of molecular size standards are shown on the left.

intracellular domain of BH-Pcdh, we performed yeast two-hybrid screening [14]. We initially used the intracellular domain of BH-Pcdh-a as bait to screen a human brain cDNA library. Although a total of 1.8×10^6 HF7c transformants were subjected to *HIS3* selection/*lacZ* screen, we could not isolate any positive clone. On the other hand, eight positive clones were identified when using the intracellular domain of BH-Pcdh-c as bait. Sequence analysis revealed that one of the isolated clones encoded PP1α and two other encoded PP1α2, which differs from PP1α by an N-terminal 11-amino-acid insert [14]. These isolated three clones encoded the entire open reading frame of PP1α and PP1α2, respectively. Others were BAI1-associated protein [17] and rat MUPP1 [18] homolog which have 5 and 13 PDZ domains, respectively, and a novel protein which showed overall 50% amino acids identity with previously reported putative G-protein, GP-1 [19], and Tip60 [20] and the rest were unknown genes.

Recent study has revealed that the PP1-binding proteins interact with the catalytic subunit of PP1 through their -(R/K)(V/I) XF-consensus motif [21]. We found a RVTF sequence that is similar to the consensus motif for PP1-binding protein at residues 1015–1018 of BH-Pcdh-c. This sequence is present only in BH-Pcdh-c because BH-Pcdh-a and -b are alternatively spliced at residues 1011 and 1012. From these observations, we selected PP1α for further experiments.

3.2. *In vitro association of PP1αwith the intracellular domain of BH-Pcdh-c*

To obtain further evidence about the interaction between BH-Pcdh-c and PP1α, we produced the intracellular domain of BH-Pcdh-c as GST fusion proteins and performed a pull-down assay using endogenous PP1α present in the lysates of human cells MKN28. C-terminus fragment of BH-Pcdh-c comprising 324 (GST-CP), 192 (GST-RVTF(+)) and 181 (GST-RVTF(−)) amino acids were expressed in *E. coli* as GST fusion protein (Fig. 1A). Equal amounts of purified GST fusion proteins and GST alone were bound to glutathione-Sepharose beads and incubated with extracts made from MKN28 cells. Pulled-down proteins were subjected to SDS-PAGE, blotted to PVDF membrane and detected with

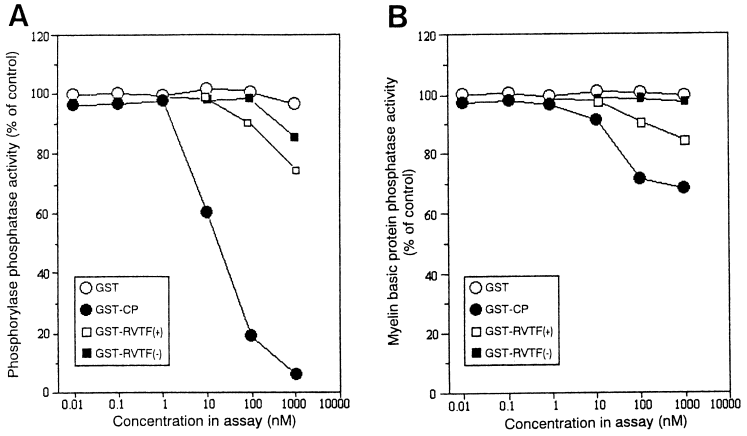

Fig. 3. Modulation of phosphatase activity by the intracellular domain of BH-Pcdh-c. Assay was performed with the catalytic subunit of PP1α and 0–1 μM range of GST, GST-CP, GST-RVTF(+) or GST-RVTF(−). Increasing concentration of GST-CP produced a potent inhibition of PP1α activity towards the phosphorylase (A) that was not seen towards myelin basic protein (B). Results were calculated as percentage of activity seen in the absence of added GST fusion protein.

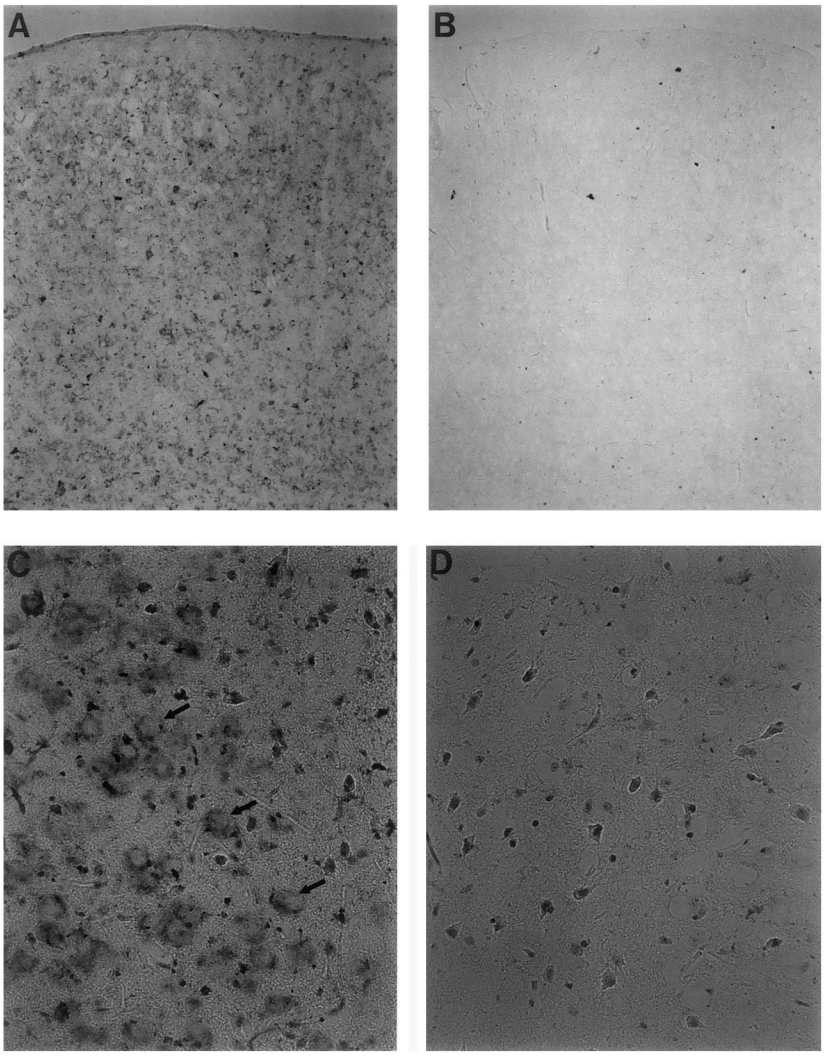

Fig. 4. Localization of BH-Pcdh-c mRNA in cerebral cortex of adult mouse brain. (A,C) In situ hybridization with an antisense BH-Pcdh-c cytoplasmic tail riboprobe (599-bp) revealed BH-Pcdh-c mRNA is predominantly expressed in cerebral cortex neurons. (B,D) As a control, mouse brain hybridized with a sense riboprobe showed no signals. The sections were incubated with alkaline phosphatase-conjugated anti-DIG antibody and visualized with NBT/BCIP. The sections were also counterstained with methyl green (C,D). Arrows indicate positive signals (C). Magnifications were 100× (A,B) and 400× (C,D), respectively.

two different anti-PP1α antibodies. PP1α was most obvious when GST-CP which encodes almost the full length of intracellular domain of BH-Pcdh-c was used for pull-down assay (Fig. 1B). In contrast, GST-RVTF (+) which encodes the residues 1009–1200 of BH-Pcdh-c including the consensus motif for PP1-binding protein showed a weak signal when compared with that of GST-CP. GST-RVTF(−), which lacks 11 amino acids including the consensus motif, showed no signal for PP1α (Fig. 1B). GST alone could not pull-down with PP1α (Fig. 1B).

From these observations, PP1α present in the lysates of MKN28 cells can associate with the intracellular domain of BH-Pcdh-c. The RVTF sequence located on the intracellular domain of BH-Pcdh-c may function as the binding site described on PP1-binding proteins.

3.3. *In vivo association of PP1α with BH-Pcdh-c*

To obtain another indication of the protein-protein interaction suggested by the two-hybrid system and in vitro pull-down results, we examined the ability of the protein product of BH-Pcdh-c to co-immunoprecipitate with PP1α in mammalian cell lysates. At first, BH-Pcdh-c was subcloned into a mammalian expression vector and expressed stably to a high level in mouse fibroblast L cells. Isolated clones showed longer shape when compared with parent or MOCK-transfected L cells (data not shown). Using these cells and an antibody directed against the C-terminus of BH-Pcdh-c, a molecular mass of approximately 160-kDa protein was observed for BH-Pcdh-c (Fig. 2C). Immunoprecipitation was carried out using this anti-BH-Pcdh-c antibody or pre-immune serum. Protein present in the immune complex was subjected to Western blotting using an anti-PP1α monoclonal antibody. The complex between PP1α and exogenous BH-Pcdh-c protein was co-immunoprecipitated with anti-BH-Pcdh-c antibody, but not with pre-immune serum (Fig. 2A). Pre-absorption of anti-BH-Pcdh-c antibody with the synthetic peptide was used for the production of antibody inhibited the co-immunoprecipitation of PP1α (Fig. 2A).

Next, we examined whether these two proteins could be co-immunoprecipitated when BH-Pcdh-c is expressed at its endogenous level in human cells. For this purpose, we used MKN28 cells because BH-Pcdh-c was originally isolated from them [13]. Immunoblot analysis showed that PP1α was found in the immunocomplex precipitated by anti-BH-Pcdh-c antibody (Fig. 2B). These results suggest that the two proteins associate with each other in mammalian cells.

3.4. Modulation of phosphatase activity by the intracellular domain of BH-Pcdh-c

To see the effect of association of BH-Pcdh-c on PP1α activity, the intracellular domain of BH-Pcdh-c was bacterially expressed as described above. Their effect on the activity of PP1α was examined using ^{32}P-labelled glycogen phosphorylase or myelin basic protein as substrate. Fig. 3A shows that GST-CP inhibited PP1α enzymatic activity towards the standard substrate, glycogen phosphorylase, normally used to assay PP1 with an IC_{50} of ~10 nM. In contrast, GST-RVTF(+) which could associate with PP1α showed only slight effect on the activity of PP1α. GST-RVTF (+) was slightly more effective than GST-RVTF(−) at higher concentrations (Fig. 3A). GST alone had no effect on the activity of PP1α at any concentrations. On the other hand, PP1α activity towards myelin basic protein was weakly suppressed by the intracellular domain of BH-Pcdh-c (Fig. 3B).

3.5. Localization of BH-Pcdh-c mRNA in cerebral cortex of adult mouse brain

Previously, we detected BH-Pcdh mRNA predominantly in the brain and heart by Northern blot analysis [13]. To determine the cellular localization of BH-Pcdh-c mRNA in the adult mouse brain, we performed in situ hybridization analysis. Using DIG-labelled antisense RNA complementary to the cytoplasmic tail region of BH-Pcdh-c as a probe, we detected BH-Pcdh-c mRNA predominantly in the cerebral cortex (Fig. 4A) and weakly in septum, thalamus, substantia nigra, pons and spinal cord. Other segments of brain such as caudoputamen, cerebellum and hippocampus did not show any signal (data not shown). In cerebral cortex, specific signal for BH-Pcdh-c mRNA overlapped with the comparatively larger nuclei, namely neurons (Fig. 4C). No specific signal was observed in adjacent sections hybridized with the sense RNA probe (Fig. 4B and D).

4. Discussion

We performed a yeast two-hybrid screen using human brain cDNA library for candidate proteins that interact with the intracellular domain of BH-Pcdh-c. We isolated eight clones including signal transduction molecules, such as PP1α, multi-PDZ domain containing protein and a novel protein containing a GTP-binding motif (data not shown). The possibility of interaction between BH-Pcdh-c and PP1α seems to be reasonable because three out of eight clones encoded the same gene product, the catalytic subunit of the serine/threonine phosphatase of type 1 (PP1) isoform α and α2. Further, BH-Pcdh-c contained the consensus binding motif -(R/K)(V/I)XF- for PP1-binding protein at residues 1015–1018 [21].

PP1 consists of a catalytic subunit with various kinds of the targeting (or regulatory) subunits, in vivo. For the catalytic subunit of PP1 (PP1c), there are 5 isoforms:α, α2,γ, γ2, and σ[14,22]. The binding protein, termed targeting (or regulatory) subunit, determines the substrate specificity and subcellular localization of PP1c [23]. At present, a number of targeting subunits have been identified and characterized [23]. Recently, PP1σ was reported to associate with focal adhesions including alpha v integrin [24]. But the binding of PP1c to a cadherin superfamily molecule has not yet been reported.

From in vitro pull-down experiments, interaction between BH-Pcdh-c and PP1α was observed when the intracellular domain of BH-Pcdh-c was fused to the GST. However, when residues 1020–1200 of BH-Pcdh-c were used for the pull-down assay, the binding of BH-Pcdh-c with PP1α was undetected. We suggest that the consensus motif for PP1-binding protein found in BH-Pcdh-c is at least required for the interaction with PP1α, although the full length intracellular domain of BH-Pcdh-c may be required for strong binding with PP1α in vitro.

The association between BH-Pcdh-c and PP1α was verified by yeast two-hybrid screening and pull-down assay. Furthermore, we performed immunoprecipitation experiment to obtain another line of evidence which suggests that the complex between BH-Pcdh-c and PP1α exists in mammalian cells. PP1α was co-immunoprecipitated from L cells expressing exogenous BH-Pcdh-c with anti-BH-Pcdh-c antibody. Moreover, PP1α presented in MKN28 cells was co-immunoprecipitated with anti-BH-Pcdh-c antibody. These results indicate that the complex of two proteins exist in mammalian cells.

The complex between PP1α and BH-Pcdh-c modulates the phosphatase activity of PP1α. The intracellular domain of BH-Pcdh-c fused to the GST inhibited PP1α enzymatic activity towards glycogen phosphorylase. On the other hand, residues 1009–1200 of BH-Pcdh-c which could associate with PP1α showed only a slight effect on the PP1α activity. PP1α enzymatic activity towards myelin basic protein was weakly suppressed by the intracellular domain of BH-Pcdh-c. The substrate-specific effect of BH-Pcdh-c on PP1α suggests that the inhibition observed could be regulatory and not a non-specific suppression of phosphatase activity.

PP1α is known to be higher expressed in brain than in other tissues [25,26]. In neurons, PP1α has been implicated in long-term synaptic plasticity [27,28]. We therefore examined the localization of BH-Pcdh-c mRNA in adult mouse brain. From in situ hybridization analysis, BH-Pcdh-c mRNA was predominantly expressed in cerebral cortex neurons. There is accumulating evidence that the classical cadherins and cadherin-related neural receptor are involved in synaptic transmission and plasticity [1,29]. Further analysis is required to understand the physiological significance of the complex between BH-Pcdh-c and PP1α.

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References

[1] Uemura, T. (1998) Cell 93, 1095–1098.
[2] Suzuki, S.T. (1996) J. Cell. Biochem. 61, 531–542.
[3] Ozawa, M., Baribault, H. and Kemler, R. (1989) EMBO J. 8, 1711–1717.
[4] Sano, K., Tanihara, H., Heimark, R.L., Obata, S., Davidson, M., St.John, T., Taketani, S. and Suzuki, S. (1993) EMBO J. 12, 2249–2256.

[5] Ozawa, M., Ringwald, M. and Kemler, R. (1990) Proc. Natl. Acad. Sci. USA 87, 4246–4250.
[6] Bradley, R.S., Espeseth, A. and Kintner, C. (1998) Curr. Biol. 8, 325–334.
[7] Kohmura, N., Senzaki, K., Hamada, S., Kai, N., Yasuda, R., Watanabe, M., Ishii, H., Yasuda, M., Mishina, M. and Yagi, T. (1998) Neuron 20, 1137–1151.
[8] Obata, S., Sago, H., Mori, N., Rochelle, J.M., Seldin, M.F., Davidson, M., St.John, T., Taketani, S. and Suzuki, S.T. (1995) J. Cell Sci. 108, 3765–3773.
[9] Sago, H., Kitagawa, M., Obata, S., Mori, N., Taketani, S., Rochelle, J.M., Seldin, M.F., Davidson, M., St.John, T. and Suzuki, S.T. (1995) Genomics 29, 631–640.
[10] Obata, S., Sago, H., Mori, N., Davidson, M., St.John, T. and Suzuki, S.T. (1998) Cell Adhes. Commun. 6, 323–333.
[11] Strehl, S., Glatt, K., Liu, Q.M., Glatt, H. and Lalande, M. (1998) Genomics 53, 81–89.
[12] Hirano, S., Yan, Q. and Suzuki, S.T. (1999) J. Neurosci. 19, 995–1005.
[13] Yoshida, K., Yoshitomo-Nakagawa, K., Seki, N., Sasaki, M. and Sugano, S. (1998) Genomics 49, 458–461.
[14] Durfee, T., Becherer, K., Chen, P,-L., Yeh, S,-H., Yang, Y., Kilburn, A.E., Lee, W,-H. and Elledge, S.J. (1993) Genes Dev. 7, 555–569.
[15] Gietz, D., St.Jean, A., Woods, R.A. and Schiestl, R.H. (1992) Nucleic Acids Res. 20, 1425.
[16] Takada, T., Lida, K., Awaji, T., Itoh, K., Takahashi, R., Shibui, A., Yoshida, K., Sugano, S. and Tsujimoto, G. (1997) Nat. Biotechnol. 15, 458–461.
[17] Shiratsuchi, T., Futamura, M., Oda, K., Nishimori, H., Nakamura, Y. and Tokino, T. (1998) Biochem. Biophys. Res. Commun. 247, 597–604.
[18] Ullmer, C., Schmuck, K., Figge, A. and Lübbert, H. (1998) FEBS Lett. 424, 63–68.
[19] Senju, S. and Nishimura, Y. (1997) Biochem. Biophys. Res. Commun. 231, 360–364.
[20] Kamine, J., Elangovan, B., Subramanian, T., Coleman, D. and Chinnadurai, G. (1996) Virology 216, 357–366.
[21] Egloff, M-P., Johnson, D.F., Moorhead, G., Cohen, P.T.W., Cohen, P. and Barford, D. (1997) EMBO J. 16, 1876–1887.
[22] Sasaki, K., Shima, H., Kitagawa, Y., Irino, S., Sugimura, T. and Nagano, M. (1990) Jpn. J. Cancer Res. 81, 1272–1280.
[23] Hubbard, M.J. and Cohen, P. (1993) Trends Biochem. Sci. 18, 172–177.
[24] Villa-Moruzzi, E., Tognarini, M., Cecchini, G. and Marchisio, P.C. (1998) Cell Adhes. Commun. 5, 297–305.
[25] Ouimet, C.C., da Cruz e Silva, E.F. and Greengard, P. (1995) Proc. Natl. Acad. Sci. USA 92, 3396–3400.
[26] da Cruz e Silva, E.F., Fox, C.A., Ouimet, C.C., Gustafson, E., Watson, S.J. and Greengard, P. (1995) J. Neurosci. 15, 3375–3389.
[27] Mulkey, R.M., Endo, S., Shenolikar, S. and Malenka, R.C. (1994) Nature 369, 486–488.
[28] Malenka, R.C. (1994) Cell 78, 535–538.
[29] Hagler Jr., D.J. and Goda, Y. (1998) Neuron 20, 1059–1062.