Molecular Cloning and Characterization of a Newly Identified Member of the Cadherin Family, PB-cadherin*

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We have isolated cDNA clones encoding novel proteins belonging to the cadherin family. These novel proteins are encoded by two distinct mRNA species generated by alternative splicing from a single gene, and based on preferential expression in the pituitary gland and brain, we named it PB-cadherin. One mRNA species encodes long type PB-cadherin composed of 803 amino acid residues with a longer cytoplasmic domain, whereas the other species encodes short type PB-cadherin composed of 694 amino acid residues with a shorter cytoplasmic domain. Both long and short type PB-cadherin contain five repeats of a cadherin motif in the extracellular domain, the transmembrane domain, and the cytoplasmic domain, and the deduced amino acid sequences have a 30% homology to those of E-, N-, and P-cadherins. Although the primary structure of N-terminal amino acids is identical between long and short type PB-cadherin, the following structures in the cytoplasmic regions are completely different. The long type PB-cadherin but not the short type contains the putative catenin-binding domain. When these two distinct forms of PB-cadherins were stably expressed in L cells, L cells expressing long type PB-cadherin or short type PB-cadherin both acquired a Ca\(^{2+}\)-dependent cell adhesion property, thereby indicating that both types of PB-cadherin are responsible for Ca\(^{2+}\)-dependent cell adhesion. Persistent expression of PB-cadherin mRNA was found in the brain of rat embryos at least from embryonic day 15 to the postnatal period. In situ localization of PB-cadherin mRNA in the adult rat brain indicated that PB-cadherin mRNA is expressed in the inner granular layer of the olfactory bulb, Purkinje cell layer of the cerebellum, and in the pineal gland. PB-cadherin may play an important role in morphogenesis and tissue formation in neural and non-neural cells for the development and maintenance of the brain and neuroendocrine organs by regulating cell-cell adhesion.

The morphogenetic process involved in cellular aggregation, segregation, and migration is mediated and controlled by a large and complex number of cell adhesion molecules that exhibit a well-regulated spatiotemporal pattern of expression during development and regeneration. Cadherins are cell adhesion molecules originally identified as a cell surface molecule responsible for Ca\(^{2+}\)-dependent cell adhesion (1). The homophilic interaction of cadherin confers cell-cell binding interaction and adhesion specificity on cells that relate to segregation, morphogenesis, neural network formation, and tumor metastasis (2-4).

Early characterization and molecular cloning revealed the presence of three distinct cadherin molecules E-, N-, and P-cadherin, in which their cell and tissue specificity and temporal expression are quite different (5-9). Cadherins are transmembrane proteins consisting of an extracellular domain that confers homophilic Ca\(^{2+}\)-dependent cell-cell binding, a transmembrane domain, and a cytoplasmic domain. The extracellular domain contains five cadherin repeat motifs and mediates calcium-dependent cell-cell interaction. The cytoplasmic domain of cadherin interacts with intracellular proteins, \(\alpha\), \(\beta\), and \(\gamma\)-catenins (10). \(\alpha\)-Catenin interacts with cytoskeletal proteins, whereas \(\beta\)-catenin is considered to regulate the function of cell-cell adhesion by tyrosine phosphorylation (11-13).

In addition to classical E-, N-, and P-cadherins, recent work (14-17) revealed that cadherin-related molecules are structurally diverse and that they constitute a cadherin superfamily. R-cadherin, B-cadherin, OB-cadherin, and cadherin 4–11 conserve a membrane spanning structure in classic cadherins. In contrast, T-cadherin lacks both the transmembrane domain and the conserved cytoplasmic domain but is attached to the plasma membrane anchored with a glycosyl phosphatidylinositol (18). Proteocadherins contain 6 or 7 extracellular repeats of the cadherin motif and the cytoplasmic domain not homologous to that of other cadherins (19). Furthermore, the Drosophila fat gene was described to be a tumor suppressor gene and contains 34 cadherin motifs in the extracellular domain; its cytoplasmic domain has no homology with that of vertebrate cadherin (20). Desmogleins, pemphigus vulgaris antigen, and desmocollins were identified as the adhesion molecule localized at the desmosome (21-25). The extracellular domain of these molecules has homology with classical cadherins, but cytoplasmic domains differ from those of classical cadherins. These diverse cadherin family molecules are thought to confer diverse cell and tissue specificities.

Involvement of cadherins in complex morphogenetic processes has been well noted, for example in neural tissue development. **This study was supported by a research grant for Science and Cancer from the Ministry of Education, Science and Culture of Japan and a research grant from Yasuda Medical Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) D83348 and D83349. **To whom correspondence should be addressed. Tel.: 81-6-879-3783; Fax: 81-6-879-3789.
opment. At the stage of neural tube closure, neural precursor cells express N- and E-cadherin, but the neural crest cells express c-cad6B, a homolog of cadherin 6, without expressing N- and E-cadherins (26). When the neural crest cells migrate outward from the neural tube, expression of c-cad6B disappears, while the cells begin to express c-cad7. Thus, in addition to a diverse repertoire of cadherin molecules, temporal expression of these cadherin superfamily molecules in a cell- and tissue-specific manner is likely to regulate cellular aggregation and segregation in a cell- and tissue-specific manner during complex morphogenetic processes.

During efforts to molecularly clone hepatocyte growth factor (HGF)-related genes (27), we isolated the cDNA clone encoding a novel protein belonging to the cadherin superfamily. The deduced amino acid sequence indicated that this cadherin has 24–51% homology with other cadherins and consists of an extracellular domain that includes five cadherin repeat motifs, a transmembrane domain, and two forms of the cytoplasmic domain. Here we report the primary structure and the localization of this novel cadherin-related molecule. Because of its predominant expression in pituitary gland and brain, we named it “PB-cadherin.”


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cDNA long type PB-cadherin and the latter short type PB-cadherin. The open reading frame begins with an ATG initiation codon at position 409 of long type PB-cadherin and at position 520 of short type PB-cadherin, in which both ATG codons are in agreement with the Kozak criteria (33). Long type PB-cadherin terminates with a stop codon at position 2848, while short type PB-cadherin at position 2602. The nucleotide sequences of 2333 bp (nucleotide 78–2410 in long type PB-cadherin and nucleotide 189–2521 in short type PB-cadherin) were completely identical in both long and short types PB-cadherin, but the following sequences (nucleotide 2411–3502 in long type PB-cadherin and 2522–4153 in short type PB-cadherin) were different. Thus, the same amino acid sequences that encode the extracellular domain, transmembrane domain, and a part of the cytoplasmic region of 23 amino acids are identical in both long and short types PB-cadherin. The poly(A) tail was not found in short type PB-cadherin, but the coding region of PB-cadherin long type is followed by 655 bp of 3'-untranslated region that contains polyadenylation signal sequences upstream of the poly(A) tail.

PB-cadherin contains the signal sequence and postulated proteolytic cleavage site of cadherin precursor polypeptides. Cleavage of the peptide at the endogenous protease cleavage site of cadherin precursor polypeptides releases the extracellular domain as a single-chain polypeptide (13). The cDNA long type PB-cadherin and the latter short type PB-cadherin were different. Thus, the same amino acid sequences that encode the extracellular domain, transmembrane domain, and a part of the cytoplasmic region of 23 amino acids are identical in both long and short types PB-cadherin. The poly(A) tail was not found in short type PB-cadherin, but the coding region of PB-cadherin long type is followed by 655 bp of 3'-untranslated region that contains polyadenylation signal sequences upstream of the poly(A) tail.

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site RXKR is one of the posttranslational modifications common to cadherins, and the RVKR site is contained in PB-cadherin (34). The deduced amino acid sequence of mature PB-cadherin exhibits structural homology with the cadherin family (Fig. 3). The extracellular domain consists of five repeats of a cadherin-specific motif, and one putative transmembrane domain is located between the extracellular domain and the cytoplasmic domain. Long type PB-cadherin has a large cytoplasmic domain and short type PB-cadherin has a small one. There are three possible N-linked glycosylation sites in the extracellular domain.

**Structural Characteristics—**Fig. 3 shows the amino acid sequence of PB-cadherin, mouse N-cadherin, mouse E-cadherin, mouse P-cadherin, mouse OB-cadherin, and chicken T-cadherin. The entire amino acid sequence of PB-cadherins has a 24–33% homology with N-, E-, and T-cadherin (Table I). PB-cadherin has 51% similarity with OB-cadherin. Because N-, E-, and P-cadherins have over 70% similarity from the mammalian to the *Xenopus*, PB-cadherin is obviously not the rat homolog of the known cadherin family and thus is a newly identified member of the cadherin family. In the extracellular five cadherin repeat motif (EC1–EC5), there are characteristic...
consensus sequences, DXD, DRE, DXNDN, that are considered to be involved in \( \text{Ca}^{2+} \) binding (35).

In the extracellular fifth cadherin motif (ECS), all four cysteine residues are conserved. In EC1 the N-terminal WV is conserved in these cadherins, except for T-cadherin. On the other hand, the HAV sequence in the EC1 domain, which is considered to confer adhesion specificity (36), is replaced with QAR in PB-cadherin, as well as OB-cadherin.

The size of the cytoplasmic domain of long type PB-cadherin is similar to those of typical cadherins. The cytoplasmic domain of long type PB-cadherin has relatively low homology with those of cadherins (<40.1%) (Table I). However, the 70-amino acid stretch at the C terminus of long type PB-cadherin has higher homology (46%) than other of those other cytoplasmic regions (Fig. 3). These homologous domains are known to be catenin-binding domains in other cadherins. In contrast, the short type PB-cadherin has only 50 amino acid residues in the cytoplasmic region, but 120 amino acid residues at the C terminus that contain catenin-binding sites are deleted. The cytoplasmic domain of the short type PB-cadherin has no significant homology with other members of the cadherin family.

Expression of PB-cadherin mRNA—We next analyzed expression of PB-cadherin mRNA in rat tissues by Northern hybridization using a \( ^{32} \text{P} \) labeled cDNA fragment of extracellular domain of PB-cadherin cDNA. The same blot was rehybridized as in A with \( ^{32} \text{P} \) labeled cDNA for rat glyceraldehyde phosphate dehydrogenase (GAPDH), as a control. The lower panels of B and C show the 18 and 28 S rRNA bands, as visualized by ethidium bromide staining.

Genomic DNA Analysis—To confirm further that both long and short types of PB-cadherin were generated by alternative splicing from a single gene, rat genomic DNA digested with restriction enzymes was hybridized with the extracellular domain of PB-cadherin as a probe. Southern hybridization revealed only one band in PstI-, EcoRl-, and EcoRV-digested genomic DNA, thereby indicating that distinct PB-cadherin mRNAs were generated from a single gene (Fig. 4).

Functional Analysis of PB-cadherins for Cell-Cell Adhesion Molecule—To identify the function of both types of PB-cadherin, their cDNA was transfected into L cells that had no endogenous cadherins or PB-cadherin. Stable transfectants were isolated by screening with G418 and cloned. The expression level of each clone was examined by RNA dot analysis, and subsequent experiments were carried out with independent clones for each PB-cadherin. Northern blot analysis showed expression of the expected sizes in the transfected cells with expression vectors for PB-cadherins (Fig. 5A). The mRNA expression of long type PB-cadherin in the transfectant cells was lower than that of short type PB-cadherin in the other transformant cells.

Each transfectant was morphologically similar to the parental cells. To examine \( \text{Ca}^{2+} \) dependent cell adhesion, a cell aggregation assay was done. Single cells treated with trypsin reaggregated in the presence of 1 mM \( \text{CaCl}_2 \), but these cells did not aggregate with trypsinization without \( \text{CaCl}_2 \) (Fig. 6). Long type PB-cadherin-transfected cells were more aggregate than the short type PB-cadherin transfectants (Fig. 5B and Fig. 6). However, the activity was not seen in the parental cells and L cells transfected with the plasmid only. These results suggest that PB-cadherin has \( \text{Ca}^{2+} \)-dependent adhesive activity, which is typical for the cadherin family.

In Situ Localization of PB-cadherin mRNA Expression in the Adult Rat Brain—To determine the cellular localization of PB-cadherin mRNA in the adult rat brain, in situ hybridization analysis was carried out, using as a probe a radio-labeled antisense RNA complementary to an extracellular domain (HincII-ClaI site) of PB-cadherin. Specific hybridization signals were observed in the inner granular layer and the glomerular layer of the olfactory bulb, anterior olfactory nucleus, primary olfactory cortex (Fig. 7A–C), Purkinje cell layer of cerebellum (Fig. 7, D and E), and pineal gland (Fig. 7F). No specific signal was observed in adjacent sections hybridized with the sense RNA probe transcribed from the same cDNA template (Fig. 7G).

In the olfactory bulb, intense hybridization was observed in the inner granular layer and the glomerular layer, whereas...
external and internal plexiform layers and the mitral cell layer were devoid of any signal. In the cerebral cortex, labeling was observed in the olfactory cortex, whereas specific hybridization signals were not detected in other cortices. Strong hybridization signals were located in the pineal gland.

In the cerebellum, dense signals were observed in the Purkinje cell layer, but the granule cell layer, molecular layer, and deep cerebellar nuclei were not labeled. No significant signals were observed in the diencephalon, mesencephalon, pons, and oblongata.

**Fig. 3. Alignment of deduced amino acid sequence with cadherins.** Rat PB-cadherin and the members of the cadherin family are aligned and numbered on the right. Residues found in all of the cadherins are marked with a dot. The boxed amino acid sequences are the cadherin motifs in the repeated extracellular domain of the cadherin family. The shaded boxes are the cysteine residues conserved among the cadherin family.
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Table I

Homology of deduced amino acid sequences in each domain between long type PB-cadherin and other cadherins

| Domain | N | E | P | OB | T |
|--------|---|---|---|----|---|
| EC1    | 32.7 | 46.6 | 30.8 | 30.8 | 21.6 |
| EC2    | 46.6 | 21.6 | 30.8 | 30.8 | 21.6 |
| EC3    | 21.6 | 21.6 | 30.8 | 30.8 | 21.6 |
| EC4    | 30.8 | 30.8 | 30.8 | 30.8 | 30.8 |
| TM     | 26.4 | 26.4 | 26.4 | 26.4 | 26.4 |
| CP     | 41.4 | 41.4 | 41.4 | 41.4 | 41.4 |
| Whole  | 38.4 | 38.4 | 38.4 | 38.4 | 38.4 |

DISCUSSION

Cadherins are involved in the morphogenesis and maintenance of tissue architecture by regulating Ca²⁺-dependent cell-cell adhesion. Distinct expression patterns of cadherins coexpressed in varying combinations in a cell- and tissue-specific manner confer segregation, segmentation, and homeostasis of the tissue architecture. In developing neural retina, early embryonic retinas incubated with antibody to N-cadherin tended to dissociate and could not be maintained as a tissue formation (37). The presence and expression of multiple types of cadherins thus enable specification of diverse tissue specificities. We cloned and characterized a novel member of cadherin, PB-cadherin, which is predominantly expressed in the pituitary gland and the brain. We also found that two types of PB-cadherin generated by alternative splicing from a single gene are functional in Ca²⁺-dependent cell adhesion.

Cadherins bind cells by means of homophilic interaction, but cadherins have a binding preference for their own type. A stretch of N-terminal 113 amino acids located in the cadherin repeat motif determines the specificity of cadherins (38). Synthetic peptides with an amino acid sequence corresponding to that of the specific binding site containing the HAV sequence can inhibit the cadherin-mediated cell-cell interactions (36). However, even though the HAV sequence is conserved in E, N, P-cadherin, heterotypic adhesion between these cadherins was nil, thus cooperation with other sites is necessary for complete binding specificity. In the EC1 domain of PB-cadherin, the HAV motif is replaced by a QAR sequence; thus, the QAR motif may be involved in the adhesive function and binding specificities of PB-cadherins.

The intracellular domain of cadherin plays a key role in cell-cell binding function through association with cytoplasmic components α-, β-, and γ-catenins (10). α-Catenin directly binds to E-, N-, and P-cadherins and intermediates the connection of cadherins and cytoskeletal proteins. Tyrosine phosphorylation of β-catenin has been found to affect the intracellular adhesion system. A specific recognition site for α-catenin is located in the C-terminal stretch comprising the 72-amino acid domain (39, 40). A comparison of the amino acid sequences of the cytoplasmic domain of long type PB-cadherin with those of the classical cadherins revealed a 33–38% sequence identity. Even in the amino acid sequence of the C-terminal 70-amino acid residues, sequence homology between long type PB-cadherin and classical cadherin was no more than 44%. These values are significantly lower than those seen within classical cadherins. Cyto-
plasmic domains of mouse E-, N-, and P-cadherins share a 57–80% homology in amino acid sequences. Recently, OB-cadherin, a new member of the cadherin family, was isolated, and the cytoplasmic domain of OB-cadherin showed only a 44–50% similarity to the classical cadherins as well as to long type PB-cadherin (16). We have yet to determine if identical catenins that associate with classical cadherins interact with PB-cadherins, but distinct homologous catenin molecules may possibly do so.

Among the cadherin superfamily, desmocollins and OB-cadherin have two distinct isoforms generated by alternative splicing (24, 25). Desmocollin III differs from desmocollin II by additional 46-bp sequences located in the cytoplasmic domain. The truncated form of OB-cadherin lacking the cytoplasmic region is generated as a splice variant, but expression in tissues and whether it is functional in cell-cell interaction are unknown. It is notable that short type PB-cadherin that lacks the distinct catenin-binding domain is synthesized as an alternative splice variant from a single gene and that this short type PB-cadherin is expressed in the pituitary gland and in the brain, at significant levels. Moreover, even though short type PB-cadherin lacks the catenin-binding domain, it is functional in the Ca\(^{2+}\)-dependent interaction. Therefore, the lack of the catenin-binding domain in short type PB-cadherin suggests that its expression may result in a constitutive homophilic binding of cells, without association of catenins by which homophilic cadherin-cadherin interaction and cytoskeletal rearrangement are regulated. Alternatively, short type PB-cadherin associates specific cytoplasmic molecules distinct from catenins. In this context, T-cadherin lacking the classical transmembrane and cytoplasmic domain is attached to the plasma membrane through a glycosyl phosphatidylinositol glycan, but T-cadherin remains functional with regard to Ca\(^{2+}\)-dependent adhesion properties (18).

In situ mRNA localization analysis showed that PB-cadherin is expressed predominantly in neurons of various regions in the adult rat brain. Localized distribution of PB-cadherin mRNA in the brain overlaps with that of N-cadherin, but cellular distribution is distinct (41). PB-cadherin mRNA expression is confined to neuronal populations in the adult rat brain. Prominent hybridization signals were detected in the olfactory bulb, primary olfactory cortex, pineal gland, and Purkinje cells of the cerebellum. The Purkinje cell is one neuron that expresses only low levels of N-cadherin, but PB-cadherin mRNA was strongly expressed. It seems to be noteworthy that PB-cadherin is also expressed in tissues responsible for neuroendocrine functions, including pineal gland and pituitary gland. PB-cadherin is also expressed in PC12 rat pheochromocytoma cells originally derived from the adrenal medulla (data not shown). The mammalian pineal gland is an endocrine component in the regulation of photoperiodic responses. The endocrine function of the pineal gland that secretes melatonin is regulated by light via the nervous system (42). In addition to the localization of PB-cadherin, PB-cadherin is expressed at high levels in fetal rat brains.

In conclusion, we cloned a novel type of cadherin, which we termed PB-cadherin. This cadherin is highly unique in its exclusive expression in the pituitary gland and in the brain, plus the presence of splicing variants. We predict that long and short types of PB-cadherin may have distinct roles, and both types of PB-cadherin may play a role in morphogenesis and tissue formation in neural and non-neural cells for the development and maintenance of the brain and neuroendocrine organs, through the potential to regulate cell-cell adhesion, and

![Fig. 7. In situ localization of PB-cadherin mRNA in adult rat brain.](http://www.jbc.org/)}
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other functions such as signal transduction. Our ongoing studies directed at spatiotemporal expression of PB-cadherin and targeted disruption of the PB-cadherin gene may elucidate biological functions of this novel member of cadherin.

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REFERENCES
1. Takeichi, M. (1991) Science 251, 1451–1455
2. Behrens, J., Mareel, M. M., Van Roy, F. M., and Birchmeier, W. (1989) J. Cell Biol. 108, 2435–2447
3. Shimoyama, Y., Hirohashi, S., Hirano, S., Nagafuchi, M., Shimosato, Y., Takeichi, M., and Abe, O. (1989) Caenorhabditis elegans directed at spatiotemporal expression of PB-cadherin and other functions such as signal transduction. Our ongoing studies directed at spatiotemporal expression of PB-cadherin and targeted disruption of the PB-cadherin gene may elucidate biological functions of this novel member of cadherin.

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