Identification of Human Cadherin-14, a Novel Neurally Specific Type II Cadherin, by Protein Interaction Cloning* 

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Cadherins, a family of Ca2+-dependent cell-cell adhesion molecules, mediate neural cell-cell interactions and may play important roles in neural development. By searching for molecules that interact with β-catenin, a cytoplasmic regulator of cadherins, we have identified a new member of the cadherin family, which we named human cadherin-14. Cadherin-14 had high amino acid sequence homology with the type II subgroup of cadherins and was broadly expressed in the central nervous system. Cadherin-14 is a novel neurally specific cell-cell adhesion molecule and may regulate neural morphogenesis.

The precise and complicated structure of the central nervous system1 is intimately associated with its functions in vivo and requires multiple cell-cell interactions. Many cell recognition molecules that mediate neuronal cell-cell interactions are implicated in neural development (1, 2). Therefore, isolating and understanding these molecules will give us a key to the mechanisms of this process.

Cadherins are a family of Ca2+-dependent cell-cell adhesion molecules (3–5). Many members of the cadherin family are expressed in the CNS and may play pivotal roles in neuronal morphogenetic events such as the elongation and targeting of axons and the segregation of neuronal precursor cells (6–9).

The classical cadherins are divided into two subgroups, called type I and type II cadherins, based on their amino acid alignments (10). Type I cadherins include E (epithelial), N (neural), P (placental), and R (retinal) cadherin, which share sequence homology with the type II subgroup of cadherins found in mouse, rat, chicken, and Xenopus (13–15). Thus this group is conserved in other species and may have important functional roles.

Cadherins are transmembrane proteins that associate with the cytoplasmic proteins, α- and β-catenin, plakoglobin, and p120, which regulate the functions of cadherins (16–18). β-Catenin also associates with APC, a colon tumor suppressor protein, and mediates cell adhesion and a growth signaling pathway (19, 20). We isolated β-catenin-associating molecules and found a new member of the cadherin family, human cadherin-14, which is a type II cadherin. Northern blot analysis revealed that cadherin-14 was broadly expressed in the CNS.

EXPERIMENTAL PROCEDURES

Production of Recombinant Protein—Using a β-catenin cDNA as a template, full-length β-catenin was amplified by PCR using the HB-1 (5′ cagggattggactatgcgaag3′) and HB-2 (5′ cagcgggtgtaaatgatcc3′) primers. The PCR product was digested with BamHI and Smal and subcloned into pGEX2TK (Pharmacia Biotech Inc.) vector. A recombinant glutathione S-transferase fusion protein was produced and purified using glutathione-coupled Sepharose 4B (Pharmacia). Purified proteins were radiolabeled in vitro with [γ-32P]ATP (ICN Pharmaceuticals, Inc.) by bovine heart kinase (Sigma) in kinase buffer (20 mM Tris, pH 7.5, 100 mM NaCl, 12 mM MgCl2).

Screening of Expression Library—One million clones of the human adult brain agt11 expression library (Clontech) were induced with 10 mM isopropyl-1-thio-β-D-galactopyranoside and transferred to nitrocellulose membranes (Amersham Corp.). These membranes were incubated with radiolabeled fusion proteins in HBB buffer (20 mM Hepes, pH 7.5, 5.5 mM MgCl2, 1 mM KCl, 5 mM dithiothreitol, 10 mM glutathione) and washed with TFBS (0.2% Triton X-100 in phosphate-buffered saline) as described previously (21). Three rounds of screening, positive phages were isolated and digested with EcoRI, and the inserts were subcloned into pBSKII-SK (Stratagene) vector for sequence analysis.

Sequencing of Cadherin-14—The nucleotide sequence of clone 36 was determined by the dyeoxy chain termination reaction using Sequenase version 2.0 (U. S. Biochemical Corp.) and dye terminator cycle sequencing kit (Perkin-Elmer), which used AmpliTaq DNA polymerase (Perkin-Elmer) and synthetic primers. Nucleotide and protein sequences were analyzed using GENETYX and the MACAW software package.

Northern Blot Analysis—The extracellular region of cadherin-14 was characterized by Northern blot hybridization using the Cad-14A (5′ gacagggcgggagagctgct3′ and Cad-14B (5′ gcacagacagggttcgtc3′) primers and [32P]dCTP (Amersham Corp.). Human β-actin cDNA was radiolabeled using Random Primed DNA Labeling kit (Boehringer Mannheim). Human multiple tissue Northern blot 1 and human brain multiple tissue Northern blots II and III (Clontech) were hybridized with radiolabeled probes (22). Filters were washed in 2 × SSC (1 × 150 mM NaCl and 15 mM sodium citrate), 0.05% sodium dodecyl sulfate for cadherin-14 cDNA and in 0.1 × SSC, 0.1% sodium dodecyl sulfate for β-actin cDNA at 65°C for 60 min as described previously (22).

RESULTS

Molecular Cloning of Human Cadherin-14—In order to isolate β-catenin-associated molecules in vitro, we performed protein interaction cloning. We screened one million clones from a human adult brain agt11 expression library using re-
diolabeled recombinant $\beta$-catenin protein and isolated 23 positive clones. Sequence analysis revealed that these clones included 11 clones of N-cadherin, 8 clones of APC, 1 clone of cadherin-11, and 3 clones of novel proteins. Sequence analysis of two novel clones, clones 18 and 36, showed that they had high sequence homology with cadherin molecules. One of the novel cadherin members, clone 36, was analyzed in this study. As 13 human cadherins had been reported previously (10), we named this clone human cadherin-14. Clone 36 has 2880 nucleotides and a poly(A) tail. When translating this sequence, we identified one long open reading frame. Comparing this amino acid sequence with other cadherins confirmed that this clone has a full coding region (Figs. 1 and 2).

Cadherin-14 Is a Type II Cadherin—The mature form of the classical cadherins has five extracellular domains of about 100 amino acids each that have high sequence homology with each other (EC-1, -2, -3, -4, and -5) (3). Type II cadherins differ from type I cadherins in the number and sequence of amino acids in each extracellular domain (11).

Amino acid alignments with other cadherins revealed that cadherin-14 was more closely related to type II cadherins (cadherin-6, 54%; cadherin-8, 58%; cadherin-11, 59%; cadherin-12, 60%) than to type I cadherins (N-cadherin, 34%; E-cadherin, 33%; P-cadherin, 32%; cadherin-4, 33%), cadherin-5 (39%), or cadherin-13 (32% in the extracellular domains) (Table I). Moreover, the deduced amino acid sequence of human cadherin-14 showed that cadherin-14 has characteristic amino acid residues that are conserved among the type II cadherins, including chick cadherin-7 and Xenopus F-cadherin (Fig. 2). In particular, the amino-terminal regions of cadherin-14 and cadherin-12 show high amino acid similarity, which may be involved in the...
specific recognition of cadherin molecules (Fig. 2 and Table I) (23). Based on these results, we conclude that cadherin-14 is a novel type II cadherin.

**The Expression of Cadherin-14 in the CNS**—We examined the expression of human cadherin-14 in human tissues. Three cadherin-14 mRNAs (9.7, 5.5, and 3.9 kb) were detected by FIG. 2. Alignment of deduced amino acid sequences of mature forms of human cadherin-14, -6, -8, -11, and -12, chicken cadherin-7, and Xenopus F-cadherin using MACAW software. Gaps (−) have been introduced to maximize the homology. The amino acid residues conserved among all of the type II cadherins are shaded. EC 1–5, extracellular subdomains 1–5; TM, transmembrane domain; CP, cytoplasmic domain.

**TABLE I**

Amino acid homology between human cadherin-14 and other cadherins for the putative mature protein

Amino acid homologies (%) between human cadherin-14 and human E-cadherin, human N-cadherin, human P-cadherin, and human cadherin-4, -5, -6, -8, -11, -12, and -13 are shown. EC 1–5, extracellular subdomains 1–5; TM, transmembrane domain; CP, cytoplasmic domain.

| EC-1 | EC-2 | EC-3 | EC-4 | EC-5 | TM | CP | Total |
|------|------|------|------|------|-----|----|-------|
| E    | 37   | 41   | 27   | 26   | 19  | 35 | 43    | 33   |
| N    | 38   | 43   | 23   | 31   | 23  | 48 | 50    | 40   |
| P    | 33   | 37   | 31   | 25   | 23  | 50 | 43    | 32   |
| 4    | 35   | 33   | 26   | 32   | 23  | 52 | 43    | 33   |
| 5    | 40   | 48   | 35   | 39   | 39  | 38 | 36    | 39   |
| 6    | 65   | 81   | 51   | 47   | 48  | 68 | 60    | 54   |
| 8    | 65   | 71   | 49   | 51   | 59  | 73 | 56    | 58   |
| 11   | 70   | 72   | 50   | 50   | 55  | 77 | 55    | 59   |
| 12   | 73   | 82   | 50   | 51   | 48  | 77 | 55    | 60   |
| 13   | 34   | 42   | 29   | 31   | 22  | 77 | 55    | 60   |

* Homology in the extracellular domains is only shown since cadherin-13 lacks transmembrane and cytoplasmic domains (30).
able by PCR. Isolating additional cadherin molecules that may be undetectable for PCR cloning. Therefore, our method can be used for general cytoplasmic amino acid sequences that differ from those virtue of its association with this is the first report of a cadherin molecule being isolated by cloning also identified a direct association between and other parts of the CNS, the signal of cadherin-14 expression in CNS. To further analyze its expression pattern within the CNS, we studied its expression by Northern blot analysis in human spinal cord, cerebellum, cerebral cortex, medulla, occipital lobe, frontal lobe, temporal lobe, putamen, and corpus callosum (Fig. 4). Three cadherin-14 mRNAs were detected in all CNS regions, but the largest mRNA (9.7 kb) was weak in the occipital, frontal, and temporal lobes. Compared with other parts of the CNS, the signal of cadherin-14 expression detected in spinal cord and corpus callosum was weak.

We also examined the expression of cadherin-14 in the amygda, caudate nucleus, substantia nigra, subthalamic nucleus, thalamus, and in the hippocampus (Fig. 4). Three mRNAs were detected in these nuclei and in the hippocampus. These results show that human cadherin-14 is broadly expressed in the human adult CNS. We also found that cadherin-14 was expressed in small-cell lung carcinoma cell lines, which have neuroectodermal cell phenotypes.

**DISCUSSION**

In this report, we have described the isolation of a novel type II human cadherin molecule. Although many cadherins and cadherin-related molecules have been isolated by PCR (10, 25), this is the first report of a cadherin molecule being isolated by virtue of its association with β-catenin. Cadherin-14 has several cytoplasmic amino acid sequences that differ from those used for PCR cloning. Therefore, our method can be used for isolating additional cadherin molecules that may be undetectable by PCR.

Recent studies have shown that there is a direct association between E-cadherin and β-catenin (26), and β-catenin also directly associates with APC, a tumor suppressor protein of human colonic cancer (19, 20). Our *in vitro* protein interaction cloning also identified a direct association between β-catenin, cadherins, and APC.

Type I cadherin (N-cadherin) and type II cadherins (cadherin-11 and -14) bind to β-catenin with equal strength *in vitro*. Post-translational modifications may regulate this association (27), but these cadherins share well conserved amino acids in the cytoplasmic β-catenin binding domain (11) so both cadherin types probably associate with catenins and function as adhesion molecules *in vivo*.

Suzuki et al. (10) reports the isolation of novel cadherin molecules from the human brain. They named these cadherins numerically from cadherin-4 to cadherin-13, and so far seven of them have been completely sequenced (11–13, 28); cadherin-4 is the human homologue of R-cadherin (29), and cadherin-13, which lacks a cytoplasmic domain, seems to be the human homologue of T-cadherin (30). Cadherin-5, -6, -8, -11, and -12 have similar structures and high amino acid homology with each other, suggesting that they form a subgroup of cadherins called type II cadherins. They share the same aromatic amino acids in their extracellular domains and have highly conserved cytoplasmic domains (11). Amino acid alignment of cadherin-14 revealed that it also shares these aromatic amino acids (Fig. 2) and that it has high protein sequence identity with the type II cadherins (Table I), suggesting that this molecule is a type II cadherin. Previously, cadherin-5 was reported to be a type II cadherin (10), but Shimoyama et al. (28) reports that cadherin-5 has low similarity with other type II cadherins. Our amino acid alignment also shows that cadherin-5 has low homology with cadherin-14 and that it might be a distinct member of the cadherin family (Table I).

Three amino-terminal amino acids of the extracellular domain, called the HAV domain, are conserved among N-, E-, P-, and R-cadherins and are thought to be essential to their specific molecular recognition (31). These amino acids are not conserved in type II cadherins and are replaced by VIV in human cadherin-5, QAI in human cadherin-6 and -14, and QAD in human cadherin-8, -11, and -12. The functional meaning of this divergence is unclear. In L-cell transfection assays, type I cadherins show homophilic adhesion properties (3). In an aggregation assay, mouse cadherin-11 has a similar homophilic

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3 T. Shibata, Y. Shimoyama, M. Gotoh, and S. Hirohashi, unpublished observation.
adhesion activity (13). Cadherin-5 shows weaker homophilic adhesion activity than cadherin-4 (12). Chick cadherin-6B and cadherin-7 show both homophilic and weak heterophilic interactions with each other (17). Therefore, whether type II cadherins have similar molecular functions to type I cadherins in vivo remains open to question. Moreover, heterophilic interactions of type I cadherins have been reported (E-cadherin and integrin αβ7/αM290β7, E-cadherin and bacterial protein, N-cadherin and fibroblast growth factor receptor) (32–35). Thus type II cadherins might be involved in a more complicated adhesion system. We are now examining whether cadherin-14 has homophilic or heterophilic cell adhesion activity.

The expression of many cadherins in the CNS and their functions in neurogenesis have been extensively studied. For example, N-cadherin, which is almost ubiquitously expressed in neural tissues, may regulate axonal growth or targeting (6, 36). R-cadherin has an expression pattern distinct from that of N-cadherin in the neural tube and may play a role in nucleus formation in the chick forebrain (37, 38). E-cadherin has various expression patterns in the brain during neuronal development. Treatment with anti-E-cadherin antibodies affects the growth pattern of the embryonic brain (39). Therefore, type I cadherins play crucial roles in neural development.

Type II cadherins were first isolated from the human brain and may have varied expression patterns in neural tissues. In Xenopus, F-cadherin, a type II cadherin, is expressed at the boundaries of the neural tube, suggesting that F-cadherin may regulate the regionalization of the neural tube in early development (15). In chick, cadherin-6B and cadherin-7 are expressed in the neural crest cells of the embryo. Chick cadherin-6B is first expressed in the presumptive neural crest cells and disappears after cell migration. Chick cadherin-7 first appears in the subpopulation of migrating neural crest cells that gives rise to dorsal and ventral root ganglia, suggesting that chick cadherin-7 may segregate migrating neural crest cells (14). The results of these studies indicate that type II cadherins play important roles in neurogenic morphogenesis, as do type I cadherins.

We detected cadherin-14 expression in cerebrum, cerebellum, and spinal cord. We analyzed various parts of the CNS to examine the specific expression of cadherin-14 and found that it was expressed in all CNS regions. Weak expression of cadherin-14 was detected in spinal cord and corpus callosum, suggesting that cadherin-14 may not be expressed at the same level throughout the CNS. It is unclear whether cadherin-14 is expressed in a distinct subgroup of neuronal cells or not. On the other hand, the expression of cadherin-14 was not detected in placenta, heart, lung, liver, skeletal muscle, kidney, and pancreas. Other type II cadherins, human cadherin-6 and -11, are expressed not only in the brain but in many other organs (28, 40). Our results indicate that cadherin-14 has a specific and fundamental role in the CNS because it is expressed throughout the CNS.

Further study of the precise expression and functions of type II cadherins, including cadherin-14, will improve our understanding of the mechanisms of neural morphogenesis.

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