Journal of Cellular Biochemistry 73:390–399 (1999)

Human p120ctn Catenin: Tissue-Specific Expression of Isoforms and Molecular Interactions With BP180/Type XVII Collagen

Sirpa Aho,* Kyle Rothenberger, and Jouni Uitto

Departments of Dermatology and Cutaneous Biology, and Biochemistry and Molecular Pharmacology, Jefferson Medical College, and Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania

Abstract Catenins, a family of structurally related proteins, are involved in epidermal keratinocyte cell-cell adhesion by interacting through their central Armadillo repeats with the intracellular domains of cadherins, transmembrane components of the adhesion junctions. p120ctn is a catenin expressed in different isoforms due to alternative splicing and multiple translation start sites. BP180 is a collagenous transmembrane protein (type XVII collagen) localized to hemidesmosomal attachment complexes in basal keratinocytes. In this study, we have delineated the molecular interaction between these two proteins utilizing the yeast two-hybrid system, which was confirmed by an in vitro protein-protein interaction assay. Specifically, it was shown that an amino-terminal segment of BP180 (aa. 13–25) contains the information necessary for binding to p120ctn isoforms 1–3, but not to the isoform 4, suggesting that the interacting domain is located immediately upstream from the Armadillo repeats and is encoded by exons 5 and 6, which are subject to alternative splicing only in a minority of transcripts. In addition to epidermal keratinocytes, p120ctn was shown to be expressed in a variety of adult and fetal tissues as well as in a number of human tumors. The expression pattern of various p120ctn transcripts, reflecting alternative splicing of the 5′ exons, was strikingly similar between the corresponding adult and fetal tissues, while the expression patterns were discordant between certain tumors and their normal parental tissues, suggesting a functional role for the tissue-specific expression of the p120ctn isoforms. Finally, the tissue-specific expression of BP180 was shown to partially overlap with that of p120ctn, suggesting that the interaction of these two proteins may contribute to the modulation of cell-cell/matrix interactions in such tissues. J. Cell. Biochem. 73:390–399, 1999. © 1999 Wiley-Liss, Inc.

Key words: BP180; p120ctn; tissue specific expression; two-hybrid; protein-protein interactions

p120ctn, a 120-kD protein, was initially identified as a substrate for phosphorylation during Src transformation of cells [Reynolds et al., 1989]. This protein has been previously referred to as p120, p120cas, and CAS, but it was recently proposed to be named p120ctn, reflecting its binding as a catenin to E-cadherin [Keirsebilck et al., 1998]. Subsequently, p120ctn was shown to be part of a complex consisting of cadherins, α-catenin, β-catenin, and plakoglobin [Reynolds et al., 1996; Staddon et al., 1995; Yap et al., 1998], and p120ctn was specifically shown to bind to the cytoplasmic domain of E-cadherin [Daniel and Reynolds, 1995; Reynolds et al., 1994; Shibamoto et al., 1995]. p120ctn, β-catenin, plakoglobin, and several recently characterized proteins belong to the family of Armadillo (Arm) proteins [Peifer et al., 1994]. Recent studies, using the yeast two-hybrid system, have confirmed the direct interaction between p120ctn and E-cadherin and have revealed that the Arm repeats 1 to 10 are necessary and sufficient for this interaction [Daniel and Reynolds, 1995].

The heterogeneous expression of p120ctn isoforms has been detected in human tumor cell lines, as well as in various murine cell lines [Mo and Reynolds, 1996]. Four of the human isoforms, designated 1–4, reflect differential utilization of a translation initiation codon due to their availability as a result of differential splic-

Grant sponsor: NIH; Grant number: P01-AR38923.

*Correspondence to: Sirpa Aho, Department of Dermatology and Cutaneous Biology, Jefferson Medical College, 233 S. 10th Street, Suite 450 BLSB, Philadelphia, PA 19107. E-mail: aho1@mail.tju.edu

Received 4 November 1998; Accepted 21 December 1998

© 1999 Wiley-Liss, Inc.

ing of the 5′-exons 1 to 6. In addition, further isoforms, designated A, B, and C, are derived from combination of alternatively spliced exons at the 3′ end of the open reading frame. No alternative splicing modifying the Armadillo repeats has been discovered, further attesting the functional importance of this region [Keirsebilck et al., 1998]. p120ctn also serves as a substrate for tyrosine kinases, and it forms a complex with a cytoplasmic kinase FER [Kim and Wong, 1995]. Only the longest isoforms of p120ctn were able to interact with FER, suggesting that the amino termini of the longer isoforms contain the sequence that is required for association with FER.

Multiple functions and subcellular localizations have been reported for various catenins. For example, β-catenin regulates gene expression by direct interaction with transcription factors, such as LEF-1 in the nucleus, providing a molecular mechanism for the transmission of signals from cell-adhesion components or from wnt protein to the nucleus [Behrens et al., 1996]. On the other hand, γ-catenin/plakoglobin interacts with desmoplakin within desmosomal structures and forms adhesion complexes with cadherins [Kowalczyk et al., 1997; Palka and Green, 1997], but nuclear localization has also been revealed [Simcha et al., 1998].

The cutaneous basement membrane zone at the dermal-epidermal junction consists of a number of adhesion structures, called hemidesmosomes, which are necessary for stable association of the epidermis to the underlying dermis [Pulkkinen and Uitto, 1998]. A key molecule in this network structure is the 180-kD bullous pemphigoid antigen (BP180), a transmembrane collagenous protein, also known as type XVII collagen. The intracellular domain of BP180 has been recently shown to interact with another hemidesmosomal adhesion molecule, β4 integrin subunit [Aho and Uitto, 1998a; Borradori et al., 1997]. The intracellular domain of β4 subunit is phosphorylated at multiple tyrosine residues in vivo, including a tyrosine-based activation motif (TAM), which is involved in the hemidesmosome assembly. The signal transduction by the α6β4 integrin is mediated by the phosphorylation of distinct tyrosine residues and recruitment of Shc/Grb2, which potentially links α6β4 to the *ras* pathway [Mainiero et al., 1995].

In this study, we demonstrate that the intracellular domain of BP180 interacts with p120ctn, and we explore the tissue-specific expression of this putative adhesion molecule in comparison to the expression of BP180.

MATERIALS AND METHODS

Yeast Strains and Vectors

The Matchmaker Two-Hybrid System (Clontech Laboratories, Inc., Palo Alto, CA) was used in this study. The two-hybrid vectors pGBT9 and pGAD424 were originally a generous gift from Dr. S. Fields (SUNY, Albany, NY). As a bait vector, pGB-*MEL1* containing the *MEL1* gene encoding α-galactosidase enzyme, was used as an independent reporter [Aho et al., 1997]. Human keratinocyte Matchmaker cDNA library was purchased from Clontech, amplified according to the manufacturer's instructions, and the two-hybrid screening was done following the manual of Matchmaker Two-Hybrid System (Clontech). As a host yeast strain, competent *Saccharomyces cerevisiae* YRG-2 cells (Stratagene, La Jolla, CA) were used. Yeast transformation was carried out according to the manufacturer's instructions. The quantitation of α-galactosidase activity was done as described by Lazo et al. [1977] with minor modifications [Aho and Uitto, 1998b].

Construction of the Fusion Vectors

The BP180 intracellular domain fragments, devoid of the transmembrane segment sequences, were produced by PCR amplification with specific primers synthesized according to the published cDNA sequence [Giudice et al., 1992] using the foreskin keratinocyte cDNA library as template. For the original bait construct (BP376), forward primer 5′-TGG ATC CTT GAA GTC ACT GAG AGA ATT GT-3′ and reverse primer 5′-GTA GCT GTG GAT ATC TGC AGT-3′ were used (the *Bam*HI site incorporated into the primer is underlined). After subcloning into the T-tailed Bluescript KS, the *Bam*HI-*Sal*I restriction fragment was isolated and ligated into the multiple cloning site of pGB-*MEL1* [Aho et al., 1997] which had been previously digested with *Bam*HI-*Sal*I. For additional bait constructs, the same forward primer shown above and one of the following reverse primers: 5′-GGG CAG GGT GGA GGA CCA-3′ (BP409), or 5′-AGT GGA GGC AGG TGA GTG-3′ (BP430), were used. For BP434, a forward primer 5′-ATG GAA TTC AGA CTT ACA TCC TTA CCA CC-3′ and the reverse primer,

5′-TCC GAG GGT ACT CCG GAG-3′ were used (the *Eco*RI site used for in-frame ligation is underlined). For the bait constructs BP455 and BP456, the pairs of oligomers consisting of two complementary strands with *Eco*RI and *Sal*I overhang ends, were annealed and ligated to the *Eco*RI-*Sal*I digested pGB-*MEL1*. The plasmids for yeast transformation were isolated using Wizard Miniprep kit from Promega (Madison, WI) and sequenced to confirm the in-frame fusion.

GST-Fusion Protein Interaction Assay

The PCR product of BP180 corresponding to aa 1–89 [Giudice et al., 1992] was produced using a forward primer 5′-A GAA TTC GGT **ATG** GAT GTA ACC AAG AAA-3′ (the *Eco* RI site used for in-frame ligation is underlined, and the first in-frame ATG of BP180 [Li et al., 1993] is shown with bold style) and a reverse primer 5′-AGT GGA GGC AGG TGA GTG-3′, which resulted in a 277-bp fragment from the keratinocyte cDNA library. After cloning into T-tailed Bluescript vector, the *Eco*RI-*Sal*I fragment was isolated and subcloned into plasmid pGEX-4T-1 (Pharmacia). Glutathione S-transferase (GST) or GST-fusion proteins were prepared from 250 ml cultures of *E. coli* BL21 cells (Promega) and purified through binding to Glutathione Sepharose 4B according to the manufacturer's (Pharmacia Biotech) instructions.

The cells from a confluent culture of primary human foreskin keratinocytes in a 100-mm dish were collected, cell lysate was prepared and incubated with the BP180-GST/GT Sepharose 4B according to the Cellular Labeling and Immunoprecipitation Kit (Boehringer, Indianapolis, IN). After careful washes, the pelleted GT-Sepharose 4B beads were boiled with an equal volume of sample buffer, proteins were resolved by SDS-PAGE, and detected by Western blotting with Anti-pp120 antibody, diluted 1:2,000 (Transduction Laboratories, Lexington, KY) or Anti-GST antibody, diluted 1:6,000 (Pharmacia). The secondary antibody, conjugated with horseradish peroxidase (HRP), was detected with Renaissance Western Blot Chemiluminescence Reagent (NEN Life Science Products, Boston, MA).

Expression Studies

Human Multiple Tissue cDNA Panels (Human MTC Panels I and II, Human Fetal MTC Panel, and Human Tumor MTC Panel) were obtained from Clontech and used as templates for PCR analysis. The $p120^{ctn}$ exon 1 specific primer, 5′-CTG CCC TGC TGG ATT TGT C-3′ (p592), and the $p120^{ctn}$ exon 7 specific primer, 5′-ATC CGA TGG CAC CTC CTC A-3′ (p593), produced a 1370-bp full length fragment and various shorter PCR products which reflect alternative splicing of the 5′ end (GenBank # AF062341) [Keirsebilck et al., 1998]. The second pair of primers, specific for the 3′-untranslated region of $p120^{ctn}$, 5′-GCT GAG TTG GCC TGG CCA-3′ (p590) and 5′-TCA TGG CTA TAC CAA TCC TCA-3′ (p591), produced a 504-bp fragment, which was used as a control for the RT-PCR analysis of the alternatively spliced transcripts. PCR conditions were: 2 min at 94°C, followed by 38 cycles of 94°C for 20 sec, 58°C for 30 sec, and 72°C for 1 min. Primers 5-CAG AAG GCG GCA TGT ATG CT-3′ and 5′-ACG GCT TGA CAG CAA TAC TTC-3′ produced a 440 bp fragment from the BP180 cDNA. The PCR conditions for BP180 were the same as for $p120^{ctn}$. G3PDH-primers, provided by Clontech with each MTC panel, were used as a control, and the PCR was performed for 30 sec at 94°C, followed by 26 cycles of 94°C for 20 sec, and 68°C for 2 min.

RESULTS

Yeast Two-Hybrid Interactions

In initial studies designed to identify molecules potentially interacting with BP180, a 1273 bp cDNA corresponding to the intracellular domain of this protein (amino acids 13–437) was cloned into the yeast two-hybrid GAL4 binding domain (BD) vector, resulting in a construct BP376 (see Fig. 1). This construct was used as a bait to screen a human keratinocyte cDNA library cloned into the GAL4 activation domain (AD) vector. Initial screening of $\sim 8 \times 10^5$ library colonies resulted in identification of several colonies capable of growing in His^- medium, suggestive of protein-protein interactions. The positive GAL4-AD plasmids were isolated and the insert-specific interactions were confirmed by re-transformation. The plasmids which consistently indicated interaction were subjected to DNA sequencing. The BLAST search [Altschul et al., 1990] against the sequences deposited in the GenBank identified one clone corresponding to the mouse p120 sequences [Reynolds et al., 1992], spanning nucleotides 66–1034. This nucleotide sequence showed 89.5% identity to the mouse cDNA, and the open reading frame of the insert in

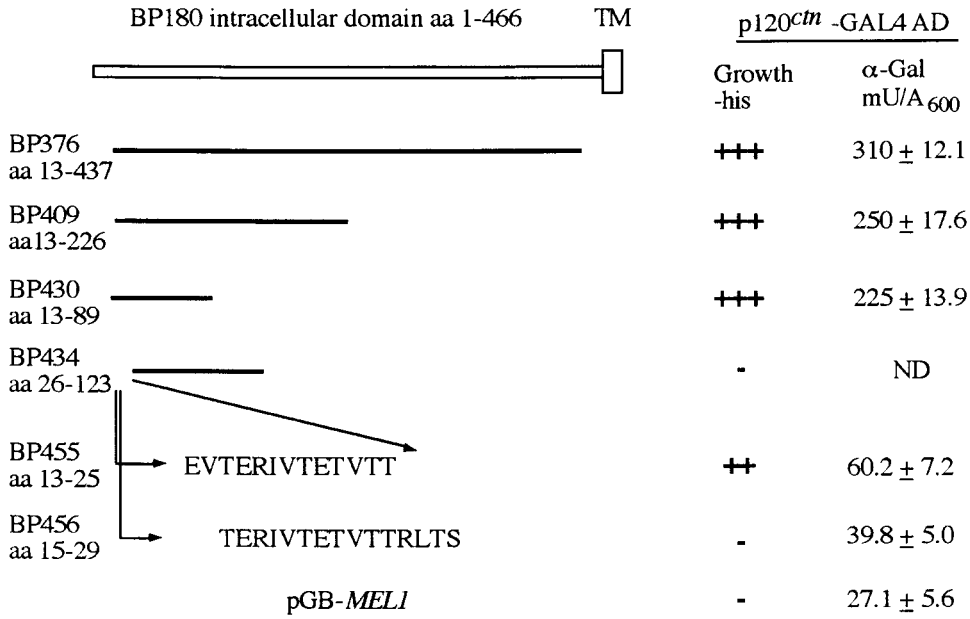

Fig. 1. The amino-terminus of BP180 interacts with p120ctn in yeast two-hybrid system. Deletion constructs of the intracellular domain of BP180 were amplified by PCR and cloned into the GAL4-binding domain vector to generate the yeast two-hybrid bait constructs BP376, BP409, BP430, and BP434, as indicated on the left. In addition, the bait constructs BP455 and BP456, with the amino acid sequences indicated, were generated using synthetic oligonucleotides. These constructs were tested for their interactions with the p120ctn-GAL4 activation domain clone obtained from the original two-hybrid screening of the Matchmaker keratinocyte cDNA library. The interactions were detected by the ability of the colonies to grow on His^- medium (+++ or ++, positive growth; −, negative growth). The bait vector contained yeast *MEL1* reporter gene encoding α-galactosidase enzyme, which was used to quantitate the interactions. The values for α-galactosidase activity are mean ± S.D. of six to 10 independent samples. ND, not determined. The pGB-*MEL1* bait cloning vector alone was used as negative control.

GAL4-AD vector revealed 97.5% identity on the amino acid level to the mouse p120. The human cDNA isolated through the two-hybrid screening is identical with the subsequently published human p120ctn, isoforms 1 and 2 [Keirsebilck et al., 1998].

To identify the region of BP180 responsible for binding to p120ctn, 3′-end deletion constructs of the cDNA, truncating the BP180 intracellular domain polypeptide at different distances from its carboxyl-terminal end, were tested, and the protein-protein interaction was quantitated by the assay of the α-galactosidase reporter enzyme. All constructs tested were found to be functional as baits (Fig. 1). However, a 5′-end deletion construct identified a discrete 13-amino acid segment, amino acids 13–25 (construct BP434) of the polypeptide as the site responsible for p120ctn binding (Fig. 1). Synthetic oligonucleotides were then used to generate a bait construct consisting of amino acids 13–25 (BP455) and 15–29 (BP456) (see Fig. 1). When co-transformed into yeast cells together with the pGAD-p120ctn, the first construct (BP455) showed low but detectable interaction, while further deletion of the two amino-terminal amino acids (BP456) totally abolished the interaction (Fig. 1). Thus, the binding of the amino-terminal segment of BP180 with the p120ctn appears to be sequence specific.

Keratinocyte-Specific Splicing Variants of p120ctn Transcript

As indicated by previous studies [Keirsebilck et al., 1998; Mo and Reynolds, 1996], p120ctn is expressed in various isoforms due to alternative splicing. To examine the presence of such isoforms in keratinocytes, PCR-analysis of a series of 10-fold dilutions of the keratinocyte cDNA library was performed. First, a pair of oligonucleotides complementary to the 3′-untranslated region of p120ctn, used as a control, showed that p120ctn is a relatively abundant transcript as compared to the G3PDH-transcript (Fig. 2). Two primers, p592 specific to exon 1 and p593 complementary to exon 7, produced from the keratinocyte cDNA four major bands, designated as K1-K4, which were approximately 960 bp, 890 bp, 820 bp, and 200 bp, respectively (Fig. 2). DNA sequencing revealed that in the transcript K1 exon 1 joined to exon 4, in the transcript K2 exon 1' joined to

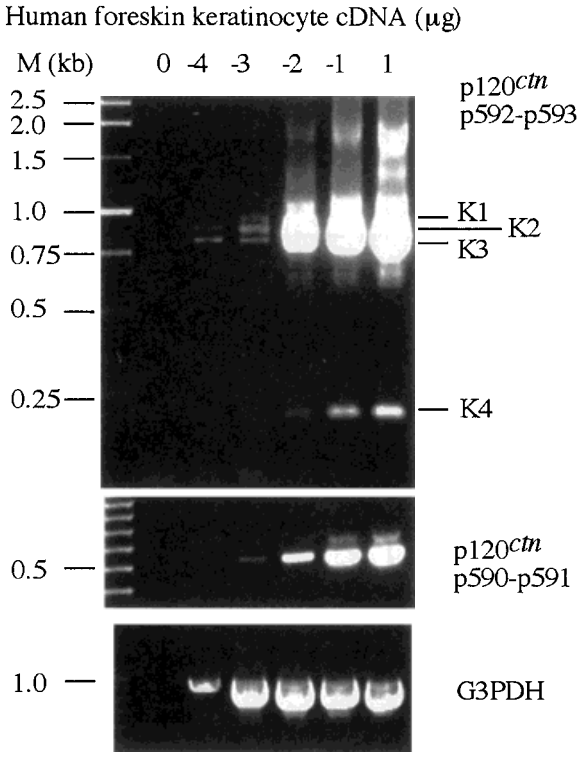

Fig. 2. The abundance of p120^{ctn} and its splicing variants in human keratinocytes. The Matchmaker human keratinocyte two-hybrid cDNA library was used as a template for PCR amplification of 10-fold dilutions (1 μg–100 pg) as indicated as log of μg on the top of the panel. The control lane (**0; left lane**) was devoid of DNA template. The primers p592-p593 amplified the region between exons 1–7 which is subject to alternative splicing. The keratinocyte specific PCR products K1-K4 are identified on the right (upper panel). Primers p590-p591 produced a 504-bp fragment from the 3′-untranslated region (middle panel). Glyceraldehyde 3-Phosphate Dehydrogenase (G3PDH), a ubiquitously expressed housekeeping gene, was amplified as a control (lower panel). The molecular weight markers (M) are shown on the left.

exon 4, in the transcript K3 exon 1' joined to the exon 5, and in the transcript K4 exon 1 joined to exon 7 (Fig. 3). Thus the bands K1-K4 correspond to previously identified isoforms 3.1, 3.3, 3.4, and 4.1, respectively [Keirsebilck et al., 1998]. Because the two-hybrid cDNA clone initially isolated by the BP180 interaction starts within exon 3, the PCR-product corresponding to that transcript may be one of the slower migrating bands noted as minor components of the PCR products (Fig. 2).

Confirmation of the BP180/ p120^{ctn} Interaction With an In Vitro Protein-Protein Binding Assay

The interaction between BP180 and the p120^{ctn} protein, initially detected in the yeast two-hybrid system, was confirmed by an independent method. Specifically, the amino-terminal region of BP180, aa 1–89, was expressed as a GST-fusion protein in *E. coli* and purified with GT-Sepharose 4B particles. The fusion protein Sepharose 4B-complex was mixed with keratinocyte cell lysate, and proteins bound to the fusion protein were analyzed by Western blotting. The monoclonal antibody pp120, which recognizes the carboxyl-terminal end of p120^{ctn}, detected from the cell lysate of cultured foreskin keratinocytes two prominent bands (bands b and c) in the size range of 90–100 kDa and a third band (band e) of 65 kDa (Fig. 4A, lanes 1 and 2). In addition, weaker bands were detectable at the approximate positions of 105 (band a) and 80 kDa (band d). Both the 90-kDa band c and the 65-kDa band e appeared to be doublets of closely migrating polypeptides (Fig. 4A).

The BP180/GST-fusion protein pull-down assay revealed interaction between the amino-terminus of BP180 and the 105-kDa, 100-kDa, and 90-kDa isoforms of p120^{ctn}, but neither the strong 65-kDa band nor the weaker 80-kDa band were detected (Fig. 4A, lane 4). No proteins interacting with GST alone were detected with the pp120 antibody (lane 5).

As a control, the GST-specific antibody was used to demonstrate the presence of bacterially expressed proteins, the 40-kDa BP180/GST fusion protein as well as its degradation products (lane 4), and the 29-kDa GST (lane 5; Fig. 4B). These findings confirm the results obtained in the yeast two-hybrid system, indicating previously undetected interactions between the BP180 and p120^{ctn}, a catenin whose direct interaction with the cell-cell adhesion molecules, cadherins, has been recently characterized [Reynolds et al., 1994] but whose interactions with the hemidesmosomal protein BP180 have not been previously disclosed.

Tissue-Specific Expression of p120^{ctn} Splicing Variants

The multiple tissue cDNA panels were used as PCR-templates to study the expression of p120^{ctn} in the adult and the corresponding fetal tissues (Fig. 5). The primers p590-p591, producing a single 504-bp band from the 3′-untranslated region of p120^{ctn}, revealed that the p120^{ctn} transcript was present in all adult and fetal tissues tested, although only a weak signal was obtained from adult skeletal muscle (Fig. 5). The 5′-primers, p592-p593, produced consistent patterns of the splicing products from the

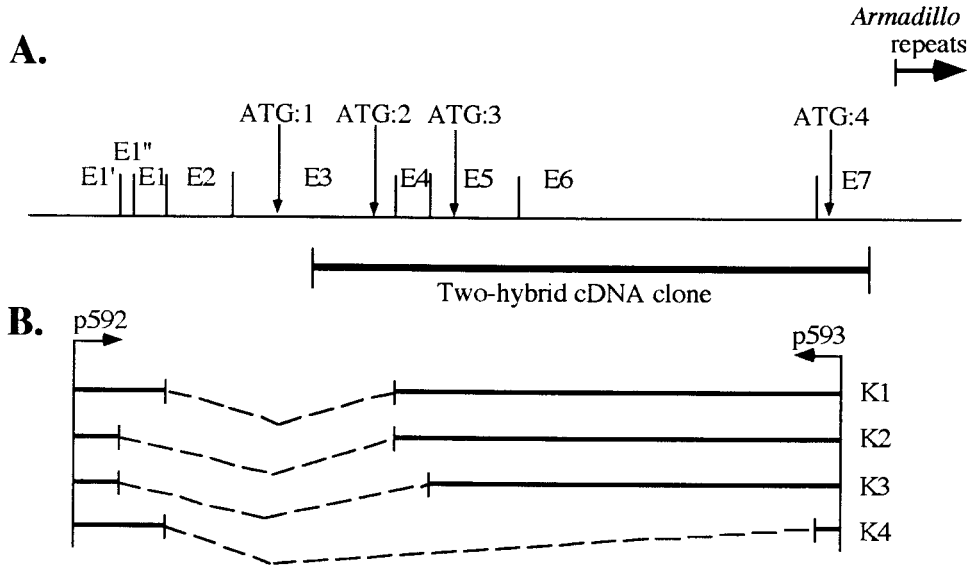

Fig. 3. Schematic representation of the alternative splicing of p120ctn transcripts in human keratinocytes. **A**: The 5′-end of the p120ctn cDNA is composed of combinations of exons 1–7, upstream from the Armadillo repeats, and the four alternative translation initiation codons (ATG:1–4) give rise to multiple p120ctn polypeptides. The positions of distinct exons (E1', E1", and E1–7, as designated by Keirsebilck et al. [1998]) are indicated. The position of the p120ctn cDNA clone isolated from the original two-hybrid screen is shown as a heavy bar. **B**: The four major keratinocyte-specific PCR-products K1-K4 generated with PCR primers p592 and p593 (see Fig. 2) were isolated and subjected to DNA sequencing. The regions deleted from each transcript are shown with broken lines.

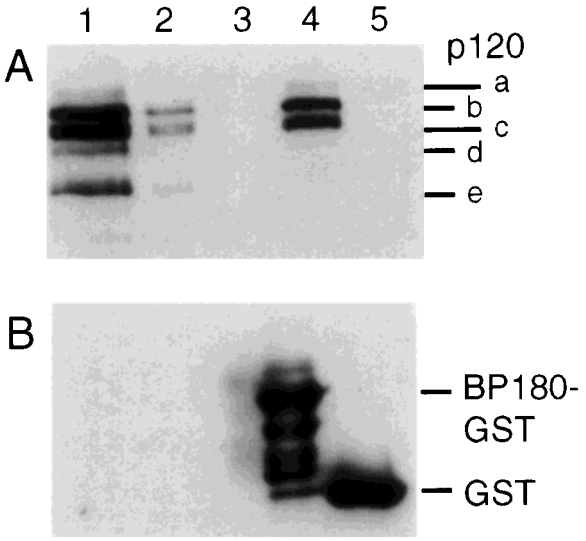

Fig. 4. The amino-terminus of BP180 interacts with specific p120ctn isoforms in in vitro protein-protein interaction assay. Cell extract of cultured foreskin keratinocytes. **Lane 1**: 20 µg; **lane 2**: 2 µg of protein; **lane 3**: blank lane; **lane 4**: keratinocyte cell extract, 100 µg, incubated with BP180/GST-Sepharose 4B; **lane 5**: incubated with GST-Sepharose 4B alone. The proteins were resolved on 10% PAGE and the Western blot was developed with an anti-p120ctn antibody (Transduction Laboratories) in **A**; and with anti-GST antibody in **B**. Note that at least five distinct bands (a–e) are noted in total keratinocyte extract (lane 1 in A), while only the three larger bands (a–c) interact with BP180 (lane 4 in A). Examination of A suggests that the bands c and e may consist of two or more individual components.

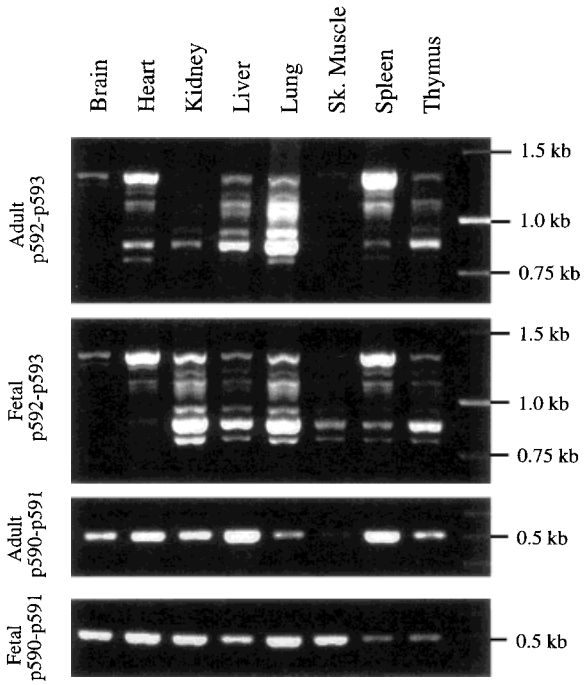

Fig. 5. Tissue-specific expression of p120ctn splicing variants in human adult and fetal tissues. The multi-tissue cDNA panels used as PCR-templates consist of cDNAs derived from polyA+ RNA isolated from tissues indicated on the top of the panel. The two top panels show the PCR-products of alternatively spliced 5′-end, corresponding to exons 1–7, in adult and fetal tissues, respectively, amplified by primers p592 and p593. In the two lower panels, amplification was performed with primers p590 and p591 which correspond to the 3′-untranslated region of p120ctn. Positions of the molecular weight markers are shown on the right.

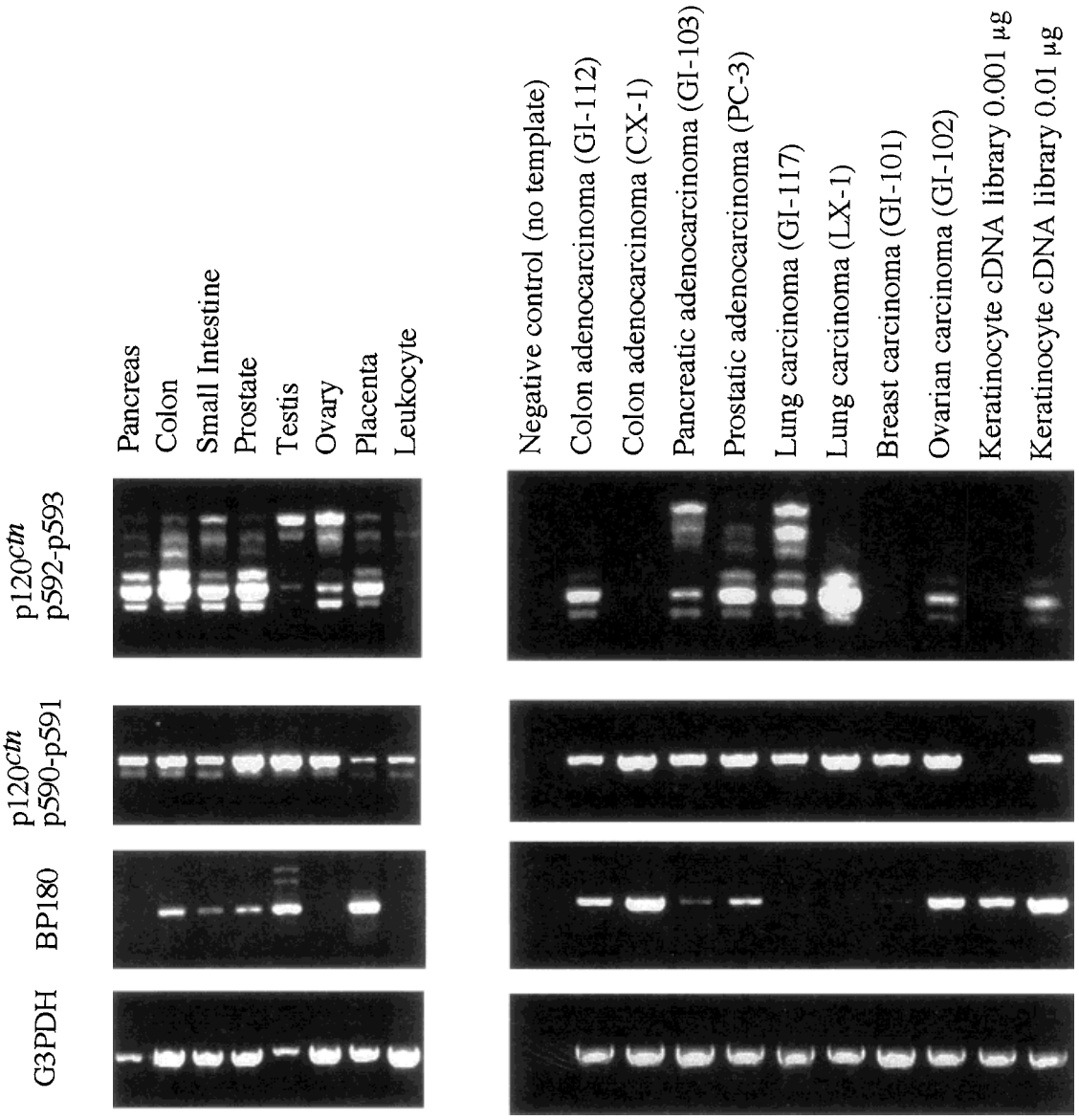

Fig. 6. Expression of p120^{ctn} and BP180 transcripts in normal and various tumor tissues. The primers p592-p593 were used to reveal the alternative splicing at the 5′-end of the gene and the primers p590-p591 corresponding to the 3′-untranslated region were used to confirm the presence of the p120^{ctn} transcript in each tissue sample. Tissue specific expression of BP180 gene is shown in the third panel, while G3PDH was amplified as a control gene (lower panel).

adult and the corresponding fetal tissues (Fig. 5).

PCR-analysis also revealed that the p120^{ctn} transcript was present in all tumor tissues studied here, but the 5′-end splicing pattern was not consistent with what was detected in the corresponding normal tissues (Fig. 6). PCR-analysis using the primers specific to exons 1 and 7 produced a 1370 bp fragment from the full-length transcript, corresponding to the isoform 1.1 [Keirsebilck et al., 1998]. DNA sequencing of selected PCR-products revealed that three isoforms, 1.1, 1.2, and 1.3 are expressed in leukocytes and only one isoform, 1.2 was present in testis. Both fetal and adult brain tissue contained isoform 1.2, but a weaker band representing isoform 1.2 devoid of exon 4 was also present. Splicing of exon 4 does not change the reading frame, but omits 24 amino acids from the translation product. It should be noted that when a specific primer corresponding to the "brain-specific" exon 1, reported by Nagase et al. [1997], was used instead of primer p592, no PCR products were detected from any of the

cells or tissues studied in Figures 5 and 6 (results not shown). The band K4 corresponding to the isoform 4.1 was detected only from keratinocytes, but a weak PCR product corresponding to isoform 4.2 was detected from most of the normal and tumor tissues (data not shown). The long transcripts retaining exons 2 and 3 were present in most of the normal tissues. It appears that either exon 1 or exon 7 is subject to alternative splicing in a colon adenocarcinoma and in a breast carcinoma tissue, because these 5′-end specific primers did not produce any PCR-product even though the 3′-primers produced a strong band from both tissues.

The two-hybrid interaction between BP180 and p120ctn was detected from foreskin keratinocyte cDNA. The multiple tissue cDNA panels were also utilized to study the expression of the BP180 transcript. While the tissues shown in Figure 5 did not express BP180 transcripts at significant level, several other tissues including colon, small intestine, prostate, testis, and placenta, as well as four adenocarcinoma and an ovarian carcinoma tissue were clearly positive for the BP180 expression (Fig. 6).

DISCUSSION

The adhesion of the epithelial basal keratinocytes to the underlying basement membrane is mediated through the adhesion structures, called hemidesmosomes, which serve as intracellular attachment sites for intermediate filaments and in the extracellular space associate with other BMZ components forming anchoring filaments [Borradori and Sonnenberg, 1996; Pulkkinen and Uitto, 1998]. BP180, also known as type XVII collagen, a transmembrane protein in type II topography, is an essential component of hemidesmosomes. In order to characterize the hemidesmosomal protein-protein interactions, we used the intracellular domain of BP180 as a bait and screened a human keratinocyte cDNA library in yeast two-hybrid system resulting in isolation of several interacting clones [Aho and Uitto, 1997]. One of the cDNA clones was highly homologous to a mouse p120ctn molecule [Reynolds et al., 1992]. When compared to the recently published human p120ctn cDNA sequence, the two-hybrid clone was found to be identical to the splicing variants, isoforms 1 and 2 [Keirsebilck et al., 1998]. The DNA sequence of the two-hybrid clone revealed that the Armadillo repeats 1 to 10, which are necessary and sufficient for the direct interaction between p120ctn and E-cadherin [Daniel and Reynolds, 1995], are not involved in the interaction between p120ctn and BP180. On the other hand, p120ctn has been shown to associate with a cytoplasmic tyrosine kinase, FER [Kim and Wong, 1995]. Since only the longest isoform of p120ctn was found to interact with FER, these results imply that the most amino-terminal end of p120ctn, encoded by exon 3, is the site of this interaction. In in vitro protein-protein interaction assay the shortest isoform, isoform 4, did not interact with BP180, suggesting that the domain responsible for this interaction is encoded by exons 5 and/or 6. Thus different domains of p120ctn are responsible for the interaction with different proteins.

In general, the amino-terminal 36 amino acids are necessary for the hemidesmosomal localization of BP180 [Borradori et al., 1997; Hopkinson et al., 1995]. Specifically, the amino-terminal domain of BP180 interacts directly with the intracellular domain of β4 integrin subunit [Aho and Uitto, 1998a; Schaapveld et al., 1998], and it was also found to interact in the yeast two-hybrid system with periplakin [Aho et al., 1998]. The two-hybrid screening has suggested direct interactions with other, less well-characterized molecules as well [Aho and Uitto, 1997]. Although computer prediction does not reveal a secretory signal sequence at the amino-terminus of the BP180 polypeptide, we omitted the first 12 amino acids of BP180 from the two-hybrid bait construct to avoid potential membrane-targeting sequences. While the bait construct containing aa 13–89 was functional, elimination of further 13 amino acids from the amino-terminus completely abolished the interaction with p120ctn. In fact, elimination of just two amino acids, Glu-13 and Val-14, was sufficient to abolish the two-hybrid interaction between BP180 and p120ctn, attesting to sequence-specificity of the two-hybrid interaction.

The multiple isoforms of p120ctn detected with a specific antibody differ from each other by the translation initiation codon usage [Keirsebilck et al., 1998]. In combination with the alternatively spliced exons, up to 32 isoforms of human p120ctn have been predicted. The isoform 1.2 devoid of exon 4, detected from brain tissue, has not been reported before. In fact, we detected five distinct bands from human keratinocytes, bands c and e being clearly doublets. In the in vitro binding assay, the BP180-GST fusion protein showed affinity to the three slower migrating bands only, suggesting that the faster migrating isoforms do not contain sequences

necessary for the interaction, again attesting to the specificity of binding.

The abundance of the longest transcripts corresponding to isoforms 1 and/or 2, appears to be low in human keratinocytes as they were not visible as distinct bands in the PCR-product, while the alternatively spliced products devoid of either exons 2 and 3 (960 bp), exons 1", 1, 2, and 3 (890 bp), or exons 1", 1, 2, 3, and 4 (820 bp) could be readily detected. A short keratinocyte-specific splicing product, devoid of exons 2–6, was detected in epidermal keratinocytes, while other tissues, including liver, pancreas, colon, fetal liver, and spleen as well as various tumor tissues showed the presence of a transcript devoid of exons 1"–6. If the latter transcripts are translated into the corresponding polypeptides, the first in-frame ATG is located in exon 7 and the translation product may correspond to the 65 kDa band detected on the Western blot of the keratinocyte extract.

PCR-analysis of the multiple tissue cDNA panels revealed that p120^{ctn} is ubiquitously expressed in a variety of fetal and adult human tissues. While the splicing pattern showed high fidelity between the comparable fetal and adult tissues, the alternative splicing pattern was considerably more heterogeneous within the tumor tissues, as also reported by recent studies by others [Keirsebilck et al., 1998; Mo and Reynolds, 1996].

Although BP180 has been characterized as a hemidesmosomal transmembrane protein which is expressed by the epidermal basal keratinocytes, the corresponding transcript was also detected in several other tissues, such as colon, small intestine, prostate, testis, placenta, thymus, fetal kidney, skeletal muscle, spleen, and thymus, and all four adenocarcinomas and an ovarian carcinoma tested. This distribution partially overlaps with that of p120^{ctn} which was found in all tissues tested. It is conceivable that p120^{ctn}/BP180 interactions could take place in any of such tissues. Finally, it is of interest to note that both p120^{ctn} [Calautti et al., 1998; Reynolds et al., 1989] and BP180 [Kitajima et al., 1995; Yamada et al., 1996] have been described both in phosphorylated and nonphosphorylated forms. It is conceivable therefore, that the phosphorylation state of these proteins alters their interactions, thus modulating the putative signaling functions of these molecules.

REFERENCES

Aho S, Arffman A, Pummi T, Uitto J. 1997. A novel reporter gene *MEL1* for the yeast two-hybrid system. Anal Biochem 253:270–272.

Aho S, McLean WHI, Li K, Uitto J. 1998. cDNA cloning, mRNA expression, and chromosomal mapping of human and mouse periplakin gene. Genomics 48:242–247.

Aho S, Uitto J. 1997. Basement membrane zone protein-protein interactions disclosed by yeast two-hybrid system. J Invest Dermatol 108:546 (Abstract).

Aho S, Uitto J. 1998a. Direct interaction between the intracellular domains of bullous pemphigoid antigen 2 (BP180) and β4 integrin, hemidesmosomal component of basal keratinocytes. Biochem Biophys Res Comm 243:694–699.

Aho S, Uitto J. 1998b. Two-hybrid analysis reveals multiple direct interactions for thrombospondin-1. Matrix Biol 17:401–412.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215: 403–410.

Behrens J, von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W. 1996. Functional interaction of β-catenin with the transcription factor LEF-1. Nature 382:638–642.

Borradori L, Koch PJ, Niessen CM, Erkeland S, van Leusden MR, Sonnenberg A. 1997. The localization of bullous pemphigoid antigen 180 (BP180) in hemidesmosomes is mediated by its cytoplasmic domain and seems to be regulated by the β4 integrin subunit. J Cell Biol 136:1333–1347.

Borradori L, Sonnenberg A. 1996. Hemidesmosomes: Roles in adhesion, signaling and human diseases. Curr Opin Cell Biol 8:647–656.

Calautti E, Cabodi S, Stein PL, Hatzfeld M, Kedersha N, Dotto GP. 1998. Tyrosine phosphorylation and Scr family kinases control keratinocyte cell-cell adhesion. J Cell Biol 141:1449–1465.

Daniel JM, Reynolds AB. 1995. The tyrosine kinase substrate p120cas binds directly to E-cadherin but not to the adenomatous polyposis coli protein or α-catenin. Mol Cell Biol 15:4819–4824.

Giudice GJ, Emery DJ, Diaz LA. 1992. Cloning and primary structural analysis of the bullous pemphigoid autoantigen, BP180. J Invest Dermatol 99:243–250.

Hopkinson SB, Baker SE, Jones JCR. 1995. Molecular genetic studies of a human epidermal autoantigen (the 180-kD bullous pemphigoid antigen/BP180): Identification of functionally important sequences within the BP180 molecule and evidence for an interaction between BP180 and α6 integrin. J Cell Biol 130:117–125.

Keirsebilck A, Bonne S, Staes K, van Hengel J, Nollet F, Reynolds A, van Roy F. 1998. Molecular cloning of the human p120ctn catenin gene (CTNND1): Expression of multiple alternatively spliced isoforms. Genomics 50:129–146.

Kim L, Wong TW. 1995. The cytoplasmic tyrosine kinase FER is associated with the catenin-like substrate pp120 and is activated by growth factors. Mol Cell Biol 15:4553–4561.

Kitajima Y, Owada MK, Fujisawa Y, Seishima M, Yaoita H, Hirako Y, Owaribe K. 1995. A hemidesmosomal transmembrane collagenous molecule, the 180-kDa bullous pemphigoid antigen (BPA II), is phosphorylated with

12-O-tetradecanoylphorbol-13-acetate in a human squamous cell carcinoma cell line (DJM-1). Epith Cell Biol 4:70–75.

Kowalczyk AP, Bornslaeger EA, Borgwardt JE, Palka HL, Dhaliwal AS, Corcoran CM, Denning MF, Green KJ. 1997. The amino-terminal domain of desmoplakin binds to plakoglobin and clusters desmosomal cadherin-plakoglobin complexes. J Cell Biol 139:773–784.

Lazo PS, Ochoa AG, Gascon S. 1977. α-galactosidase from *Saccharomyces carlsbergensis*. Eur J Biochem 77:375–382.

Li K, Tamai K, Tan EML, Uitto J. 1993. Cloning of type XVII collagen. Complementary and genomic DNA sequences of mouse 180-kilodalton bullous pemphigoid antigen (BPAG2) predict an interrupted collagenous domain, a transmembrane segment, and unusual features in the 5′-end of the gene and the 3′-untranslated region of the mRNA. J Biol Chem 268:8825–8834.

Mainiero F, Pepe A, Wary KK, Spinardi L, Mohammadi L, Schlessinger J, Giancotti FG. 1995. Signal transduction by the α6β4 integrin: Distinct β4 subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes. EMBO J 14:4470–4481.

Mo Y-Y, Reynolds AB. 1996. Identification of murine p120^{cas} isoforms and heterogenous expression of p120^{cas} isoforms in human tumor cell lines. Cancer Res 56:2633–2640.

Nagase T, Ishigawa K-I, Nakajima D, Ohira D, Seki N, Miyajima N, Tanaka A, Kotani H, Nomura N, Ohara O. 1997. Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. DNA Res 4:141–150.

Palka HL, Green KJ. 1997. Roles of plakoglobin end domains in desmosome assembly. J Cell Sci 110:2359–2371.

Peifer M, Berg S, Reynolds AB. 1994. A repeating amino acid motif shared by proteins with diverse cellular roles. Cell 76:789–791.

Pulkkinen L, Uitto J. 1998. Hemidesmosomal variants of epidermolysis bullosa. Mutations in the α6β4 integrins and the 180-kD bullous pemphigoid antigen/type XVII collagen genes. Exp Dermatol 7:46–64.

Reynolds AB, Daniel J, McCrea PD, Wheelock MJ, Wu J, Zhang Z. 1994. Identification of a new catenin: The tyrosin kinase substrate p120cas associates with E-cadherin complexes. J Cell Biol 14:8333–8342.

Reynolds AB, Daniel JM, Mo Y-Y, Wu J, Zhang Z. 1996. The novel catenin p120^{cas} binds classical cadherins and induces an unusual morphological phenotype in NIH3T3 fibroblasts. Exp Cell Res 225:328–337.

Reynolds AB, Herbert L, Cleveland JL, Berg SB, Gaut JR. 1992. p120, a novel substrate of protein tyrosine kinase receptors and of p60$^{v\text{-}src}$, is related to cadherin-binding factors β-catenin, plakoglobin and *armadillo*. Oncogene 7:2439–2445.

Reynolds AB, Roesel DJ, Kanner SB, Parsons JT. 1989. Transformation-specific tyrosine phosphorylation of a novel cellular protein in chicken cells expressing oncogenic variants of the avian cellular src gene. Mol Cell Biol 9:629–638.

Schaapveld RQJ, Borradori L, Geerts D, van Leusden MR, Kuikman I, Nievers MG, Niessen CM, Steenbergen RDM, Snijders PJF, Sonnenberg A. 1998. Hemidesmosome formation is initiated by the β4 integrin subunit, requires complex formation of β4 and HD1/plectin, and involves direct interaction between β4 and the bullous pemphigoid antigen 180. J Cell Biol 142:271–284.

Shibamoto S, Hayakawa M, Takeuchi K, Hori T, Miyazawa K, Kitamura N, Johnson KR, Wheelock MJ, Matsuyoshi N, Takeichi M, Ito F. 1995. Association of p120, a tyrosine kinase substrate, with E-cadherin/catenin complexes. J Cell Biol 128:949–957.

Simcha I, Shtutman M, Salomon D, Zhurinsky J, Sadot E, Geiger B, Ben-Ze'ev A. 1998. Differential nuclear translocation and transactivation potential of β-catenin and plakoglobin. J Cell Biol 141:1433–1448.

Staddon JM, Smales C, Schulze C, Esch FS, Rubin LL. 1995. p120, a p120-related protein (p100), and the cadherin/catenin complex. J Cell Biol 130:369–381.

Yamada T, Endo R, Tsukagoshi K, Fujita S, Honda K, Kinoshita M, Hasebe T, Hirohashi S. 1996. Aberrant expression of a hemidesmosomal protein, bullous pemphigoid antigen 2, in human squamous cell carcinoma. Lab Invest 75:589–600.

Yap AS, Niessen CM, Gumbiner BM. 1998. The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening, and interaction with p120^{ctn}. J Cell Biol 141:779–789.