Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 10330–10334, September 1997
Medical Sciences

β-Catenin mutations in cell lines established from human colorectal cancers

(sequencing/CTNNB1/sequencing/intestinal cancer)

M. ILYAS*†‡, I. P. M. TOMLINSON†§, A. ROWAN*, M. PIGNATELLI¶, AND W. F. BODMER*

*Cancer Genetics and Immunology Laboratory, Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom; §The Haddow laboratories, Institute of Cancer Research, The Royal Marsden Hospital, Sutton, Surrey, SH2 5NG, United Kingdom; and ¶Department of Pathology, Royal Post-Graduate Medical School, Hammersmith Hospital, Du Cane Road, London WI2 0HS, United Kingdom

Contributed by Walter Bodmer, July 3, 1997

ABSTRACT **β-catenin has functions as both an adhesion and a signaling molecule. Disruption of these functions through mutations of the β-catenin gene (*CTNNB1*) may be important in the development of colorectal tumors. We examined the entire coding sequence of β-catenin by reverse transcriptase–PCR (RT-PCR) and direct sequencing of 23 human colorectal cancer cell lines from 21 patients. In two cell lines, there was apparent instability of the β-catenin mRNA. Five different mutations (26%) were found in the remaining 21cell lines (from 19 patients). A three-base deletion (codon 45) was identified in the cell line HCT 116, whereas cell lines SW 48, HCA 46, CACO 2, and Colo 201 each contained single-base missense mutations (codons 33, 183, 245, and 287, respectively). All 23 cell lines had full-length β-catenin protein that was detectable by Western blotting and that coprecipitated with E-cadherin. In three of the cell lines with *CTNNB1* mutations, complexes of β-catenin with α-catenin and APC were detectable. In SW48 and HCA 46, however, we did not detect complexes of β-catenin protein with α-catenin and APC, respectively. These results show that selection of *CTNNB1* mutations occurs in up to 26% of colorectal cancers from which cell lines are derived. In these cases, mutation selection is probably for altered β-catenin function, which may significantly alter intracellular signaling and intercellular adhesion and may serve as a complement to *APC* mutations in the early stages of tumorigenesis.**

β-catenin is one of a large group of functionally diverse proteins containing *Arm* repeat sequences (1). The *Arm* repeats consist of 42 amino acids and are thought to act as molecular adapters that help to coordinate the actions of these molecules (2). Human β-catenin is a 92-kDa protein (3) and contains 13 *Arm* repeats. It shows a high degree of evolutionary conservation and shares more than 80% homology with the Armadillo protein in *Drosophila* and almost complete homology with murine β-catenin (2). Although all the functions of human β-catenin are not known, many can be surmised from the known functions of its homologues in *Xenopus laevis* and *Drosophila* (2, 4, 5). In these species, the proteins have a role in signaling and are essential for embryonic development and tissue organization. Inhibition of the protein results in failure to develop normal tissue architecture, and forced overexpression of the proteins results in tissue reduplication (6–9). A role for β-catenin, in both humans and *Xenopus*, that may contribute to its higher organizational functions is the control of intercellular adhesion. It can complex with E-cadherin and α-catenin to form a functional unit called the E-cadherin–catenin unit, which is located at the sites of *adherens* junctions (4, 10).

In the cell, β-catenin can be localized to both the cytoplasm, where it exists as pools of free monomeric protein, and to the lateral cell membranes as part of the E-cadherin–catenin unit. β-catenin protein has also been detected in the nucleus, where it has been shown to complex with transcription factors such as pangolin (11) in *Drosophila* to initiate transcription of genes such as *engrailed* (5, 12). In humans, β-catenin appears to complex with the Lef-Tcf family of transcription factors (human homologues of pangolin), although the target genes are not yet known (12, 13). Regulation of the level of free cytoplasmic β-catenin is, in part at least, through the opposing actions of the Adenomatous Polyposis Coli (*APC*) onco-suppressor gene and the Wnt-1 proto-oncogene. APC protein, which also contains Arm repeats, appears to serve as a negative regulator of cytoplasmic β-catenin (14), whereas expression of *Wnt 1*, the human homologue of the Wingless gene in *Drosophila*, is associated with increased levels of free cytoplasmic β-catenin (15, 16).

The *APC* gene is the most commonly mutated gene in colorectal cancer. Many mutations result in a truncated protein with loss of the β-catenin regulatory activity. In addition, nuclear expression of β-catenin, which in normal colonic tissue is below the threshold of immunohistochemical detection, has been reported in colorectal polyps (17). Mutation of the β-catenin gene, *CTNNB1,* would result in disruption of a large number of cellular functions that may be important in tumor development. We have previously published preliminary data (18) from 23 colorectal cancer cell lines in which we screened for mutations in *CTNNB1*. Here, we report these data more fully and discuss the effects of *CTNNB1* mutations on β-catenin function. While this study was in progress, similar observations were published by Morin *et al.* (21).

MATERIALS AND METHODS

Cell Lines. The cell lines examined are given in Table 1. All the cell lines contained mutant APC protein except SW 48, HCT 116, LS174T, and HCA 7. The APC status of HCA 46 is, as yet, uncertain. The cells were grown either in DMEM (Imperial Cancer Research Fund) with 10% fetal calf serum or RPMI 1640 medium (Sigma) with 20% fetal calf serum at an atmospheric carbon dioxide content of 10% and 5%, respectively. Three of the cell lines (Colo 201, Colo 205, and Colo 320) were semi-adherent with both a floating component and an adherent component. There were two pairs of cell lines (Colo 201/Colo 205 and SW 480/SW 620) in which one of the pair was developed from the primary tumor and the other was from a metastasis of the same tumor. Cells were grown to confluence and then collected for mRNA extraction. For the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: SSCP, single-strand conformation polymorphism.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. Z19054).

†M.I. and I.P.M.T. contributed equally to this manuscript.

‡To whom reprint requests should be addressed. e-mail: ilyas@europa.lif.icnet.uk.

Table 1. List and *APC* gene status of cell lines examined

Cell line	APC status
Colo 201*	Mutant
Colo 205*	Mutant
Colo 320	Mutant
DLD 1	Mutant
SW 1222	Mutant
SW 832	Mutant
HT 29	Mutant
LS 174T	Wild type
SW 403	Mutant
CACO 2	Mutant
SW 480+	Mutant
SW 620+	Mutant
SW 948	Mutant
HCA 7	Wild type
HRA 19	Mutant
HCT 116	Wild type
LOVO	Mutant
JW	Mutant
HCA 46	Uncertain
SW 48	Wild type
CCO7	Mutant
SW 1417	Mutant
SKCO 1	Mutant

The *APC* status of the cell line HCA 46 is currently unknown. Mutations have not been detected by the Protein Truncation Test for exon 15 and neither wild-type nor truncated APC protein is detectable by Western blotting. * and + indicate origin from the same patient. (References for these cell lines are available from the authors.)

semi-adherent cell lines, the floating component was collected first for separate analysis from the adherent component.

mRNA Extraction and reverse transcriptase–PCR (RT-PCR). mRNA was extracted from approximately 1×10^7 cells using the QuickPrep Micro mRNA purification kit (Pharmacia) as per the manufacturer's instructions. cDNA was made from the extracted mRNA using the First-Strand cDNA Synthesis Kit (Pharmacia). A mixture containing 1 μl DTT solution (200 mM) and 1 μl pd$(N)_6$ primer (random hexanucleotides at 0.2 $\mu g \cdot \mu l^{-1}$) added to the mRNA solution was incubated for 1 hr at 37°C. PCR was then performed using 2–3 μl of this solution as template.

The entire coding sequence for β-catenin was examined as three separate regions. Primers for each of the regions (and all other reactions) were designed using the Oligo primer design program (National Biosciences, Plymouth, MN). All reactions were carried out in a total volume of 50 μl with final reaction concentrations of 1× standard PCR buffer (Promega), 200 μM dNTPs, 0.4 μM each primer, and 1.5 mM Mg^{2+} with 2–3 μl cDNA. Identical cycling conditions were used for all primer pairs and these comprised an initial denaturing step of 96°C for 3 min, 35 cycles of 96°C × 1 min/52°C × 1 min/72°C × 1 min, with a final extension step of 72°C for 5 min.

In those cases in which there was unsuccessful amplification of β-catenin, the integrity of the extracted cDNA was tested using primers designed for the cDNA of the human Secretory Phospholipase A2 gene and the Tissue Plasminogen Activator gene. The cycling conditions were as for β-catenin, except that an annealing temperature of 55°C was used rather than 52°C.

To ensure that the missense mutations detected were not polymorphisms, primers were designed for exons 3, 5, and 6 (in which the missense mutations were found). PCR was performed on 30 samples of genomic DNA prepared from the peripheral blood of human random controls and on genomic DNA from the cell lines with the mutations. Apart from the annealing temperature and magnesium concentration, which were optimized for each primer pair, the cycling conditions were identical to those used for β-catenin. Sequences for all the primers used are available from the authors upon request.

DNA Sequencing. The products of each PCR were purified from oligonucleotides and unincorporated dNTPs using a Sephadex G50 spin column (Pharmacia). Five microliters of purified PCR product were used in a thermocycle sequencing reaction with the Ready Reaction Dye Terminator Cycle Sequencing kit (Applied Biosystems). Polyacrylamide gel electrophoresis was performed using standard conditions on a 377 Prism fluorescence-based, semi-automated DNA sequencer (Applied Biosystems). All sequencing reactions were performed using both the forward and reverse primers for each of the β-catenin regions. The PCR and sequencing reactions were repeated in all cases in which a mutation was found to confirm the presence of the mutation and exclude PCR errors. The sequences obtained from our experiments were compared with the published sequences of β-catenin (GenBank accession no. Z19054) using the MULTIPLE sequence analysis software from the Wisconsin package (Genetics Computer Group, Madison, WI). Once the mutations were confirmed, we assessed their presence in functional domains by scanning the β-catenin amino acid sequence for functional motifs using the MOTIF program from the Wisconsin package.

Single-Strand Conformation Polymorphism (SSCP). SSCP analysis was carried out on the PCR products for exons 3, 5, and 6 to exclude the possibility that the mutations we had detected were polymorphisms. Three microliters of PCR product were mixed with 4 μl of loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured by boiling, and immediately placed on ice. The mixture was run overnight at 20 mA on a 6% polyacrylamide gel containing 10% glycerol. The gel was fixed in a solution containing 10% ethanol and 0.5% acetic acid and stained by soaking for 15 min in a 0.1% solution of silver nitrate. After two quick washes in distilled water, the gel was incubated in a solution of 1.5% sodium hydroxide and 0.1% formaldehyde to visualize the bands. The gel was dried and inspected for any band shifts of the random human samples compared with the cell lines with the mutations (which were used as positive controls).

Immunoprecipitation and Western Blotting. Further immunoprecipitation studies to detect any changes in complex formation of β-catenin with α-catenin, E-cadherin, and APC were carried out on those cell lines in which mutations were discovered. Cells were grown to confluence in 10-cm Petri dishes, and protein was extracted from the monolayers with ice-cold lysis buffer (0.5% Mega 9, 0.5% NP40, 150 mM sodium chloride, 5 mM EDTA, 50 mM Tris, 2 mM phenylmethysulfonyl fluoride, 5 mM iodoacetamide). One milliliter of buffer was added to each dish followed by 15 min of agitation on ice. The mixture was collected and centrifuged at 14,000 rpm to remove the insoluble material. The supernatant was separated and mixed with 2 μg of anti β-catenin antibody (Affinity, Nottingham, U.K.) for 1 hr at room temperature. Fifty microliters of anti-IgG Sepharose (Pharmacia) was added and allowed to mix for 30 min. The Sepharose was washed five times in PBS and mixed with 50 μl sample buffer (50 mM Tris·HCl, pH 6.8/4% SDS/20% sucrose/0.05% bromophenol blue/0.1 M DTT). This was boiled and run on a 10% SDS/polyacrylamide resolving gel at 180 V. Rainbow markers (Amersham) were run with each gel. Protein was blotted onto Electran nitrocellulose membranes (BDH) by electrophoretic transfer. For immunoprecipitation with APC, the Sepharose was run on a 3% low-melting-point agarose gel (GIBCO/BRL), and protein was blotted onto the membrane by capillary transfer. The membranes were washed for 1 hr in a 3% solution of Marvel dried milk in PBS and incubated overnight at room temperature with the primary mouse monoclonal antibody [α-catenin (Affinity), 1:1,000; HECD-1 (ICRF), undiluted supernatant; APC (Imperial Cancer Research Fund, unpublished work), undiluted supernatant]. The membranes were

then washed five times in 3% Marvel in PBS and then incubated with 1:10,000 dilution of horseradish peroxidase-labeled rabbit anti-mouse (Dako) for 1 hr. Bound antibody was detected using the enhanced chemiluminescence (ECL) method (Amersham) and exposing the membranes to XAR film (Kodak) for 2–5 min before developing.

In two cell lines, CCO7 and SW1417, no β-catenin mRNA was detected. These were examined for the presence of β-catenin protein. The methods were as above except that, after extraction from the cell monolayer, 150 μl of protein was mixed with 50 μl sample buffer and run on a 10% resolving gel. After blotting, the protein was detected using anti-β-catenin antibody (Affinity) and the ECL method.

RESULTS

Sequence Analysis. The results of the sequence analysis together with the predicted amino acid changes and *APC* status are shown in Fig. 1 and Table 2. Five mutations were detected in total. In SW 48 there was a heterozygous C-to-A missense transversion mutation in codon 33 in exon 3. This would be predicted to cause a change from serine to tyrosine, thereby replacing one putative phosphorylation site with another and changing to a more hydrophobic residue (Fig. 1*b*). HCT 116 had a heterozygous three-base-pair deletion of codon 45 in exon 3, resulting in loss of a serine residue. In both HCT 116 and SW 48 the mutations were in a region outside the *Arm* repeats 5′ to the α-catenin binding site, and both the serine residues that were mutated are conserved from *Drosophila* to humans. This region's function is as yet unknown and may form the target for the serine/threonine kinase GSK3 β (19).

In HCA 46 there was a heterozygous G-to-T missense transversion mutation in codon 183 in exon 5, causing a change from alanine to serine (Fig. 1*b*). This mutation lies within the second *Arm* repeat, replacing a nonpolar amino acid with a polar amino acid, and introduces a new potential site for serine phosphorylation. $CACO_2$ had a heterozygous G-to-C missense transversion mutation in codon 245 in exon 5, causing a change from glycine to alanine (Fig. 1*b*). This mutation lies within the third *Arm* repeat and would cause little change in polarity or hydrophobicity at that site but would increase the accessible surface area at that site (20). The primary structure changes in both HCA 46 and $CACO_2$ may affect phosphorylation of nearby serine residues through changes in target recognition sequences and tertiary protein structure.

The cell lines Colo 201 and Colo 205 are derived from the primary tumor and a metastasis in the same patient. These cell lines grow as semi-adherent lines, and the adherent and floating components were examined separately. Both components of both of the cell lines showed a homozygous A-to-G missense transition mutation in codon 287 in exon 6, and no splice variants were identified. This would cause a change from asparagine to serine (Fig. 1*b*), although it is uncertain whether the new serine residue would be available as a target for phosphorylation. The mutation lies in the fourth *Arm* repeat in a region of β-catenin that complexes with both APC and E-cadherin.

In two of the cell lines, CCO7 and SW1417, there was no amplification when using the above primers for β-catenin despite repeated mRNA extraction. We tested the integrity of the cDNA made from these cell lines with primers designed for cDNA for the Secretory Phospholipase A2 and Tissue Plasminogen Activator genes. Both of these cell lines showed successful amplification with these primers (data not shown).

SSCP. The regions showing the mutations were examined by SSCP, and bandshifts were seen in the cell line samples but not in any of the random DNA samples (data not shown). This confirmed that the mutations were not simply germ-line polymorphisms.

Immunoprecipitation and Western Blotting. Immunoprecipitation studies in all the cell lines with *CTNNB1* mutations showed bands of the appropriate size, with the antibody for

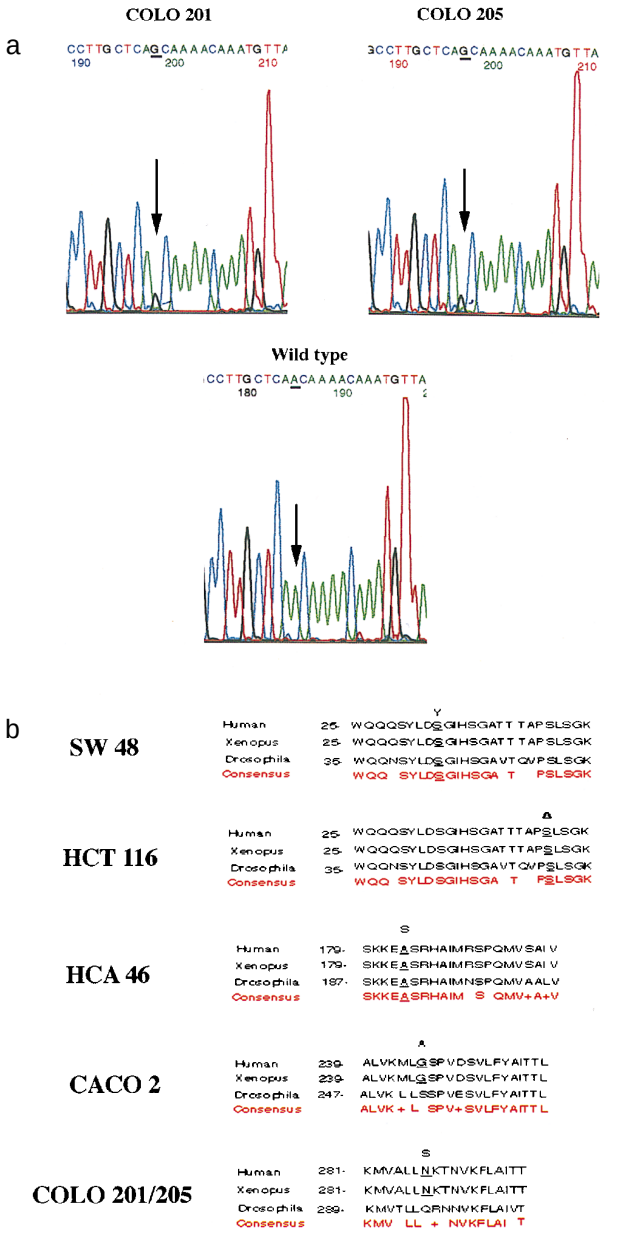

FIG. 1. (*a*) Sequence analysis for the cell lines Colo 201 and 205 with the corresponding wild-type sequence. These cell lines are derived from the primary and metastatic tumor in the same patient and show a missense mutation in codon 287 (indicated by arrow), which results in an A-to-G substitution. The mutation is homozygous and this case demonstrates that *CTNNB1* mutation and allelic loss occurred before tumor metastasis. (*b*) The effects of the mutations on the primary structure of β-catenin and the level of evolutionary conservation of the domains in which the mutations are occurring. In SW 48 the missense mutation results in substitution of a highly conserved serine residue (from *Drosophila* to man) to a tyrosine residue. In HCT 116, a three-base deletion results in loss of a similarly conserved serine residue. HCA 46 shows substitution of a highly conserved alanine residue (from *Drosophila* to man) by a serine residue as a result of a missense mutation. In CACO 2 and Colo 201/205, a missense mutation results in substitution of alanine for glycine and serine for asparagine, respectively, in residues that are conserved from *Xenopus* to man.

E-cadherin demonstrating the presence of β-catenin/E-cadherin complexes (Fig. 2*a*). We were able to demonstrate the presence of β-catenin/α-catenin complexes in all cell lines except SW 48 (Fig. 2*b*) and β-catenin/APC complexes were detectable in all cell lines except HCA 46 (Fig. 2*c*). HCA 46 is rather an unusual cell line inasmuch as there is no mutation

Table 2. Mutations found in the *CTNNB1* gene

Cell line	Mutation	Mutation status	Predicted change	Site of mutation	APC status
SW48	C → A	Heterozygous	Serine → tyrosine	Exon 3 (codon 33)	Wild type
HCT 116	Three-base deletion	Heterozygous	In-frame deletion	Exon 3 (codon 45)	Wild type
HCA 46	G → T	Heterozygous	Alanine → serine	Exon 5 (codon 183)	Uncertain
Caco 2	G → C	Heterozygous	Glycine → alanine	Exon 5 (codon 245)	Mutant
Colo 201/205	A → G	Homozygous	Asparagine → serine	Exon 6 (codon 287)	Mutant

detectable in exon 15 by the Protein Truncation Test but neither wild-type nor truncated protein has been detected by Western blotting. We are as yet uncertain as to whether this is due to very low levels of wild-type protein or a mutation at the 5′ part of the gene resulting in an unstable truncated protein or unstable message.

In the cell lines CCO7 and SW1417, there was no amplification using the above primers despite repeated mRNA extraction. Both of these cell lines contained β-catenin protein (Fig. 2*d*), indicating that despite mRNA instability, protein synthesis was still occurring.

DISCUSSION

β-catenin is involved in both intracellular signaling and cell adhesion. Mutation of the β-catenin gene *CTNNB1* presumably disrupts these functions, leading to loss of growth control and neoplastic change. Many of the activities of β-catenin are regulated through control of functional complexes with other molecules (such as APC, E-cadherin, and α-catenin) or phosphorylation of β-catenin by protein kinases (such as epidermal growth factor receptor). We investigated 23 colorectal cancer cell lines derived from cancers from 21 patients for the presence of mutations in *CTNNB1*. Only 21 of these had stable β-catenin mRNA, and in these there were 5 *CTNNB1* mutations. Because each of two pairs of cell lines was derived from the primary and metastatic tumor in the same patient, the overall frequency of *CTNNB1* mutation was 26%. We have previously described four of these mutations (18). A recent study by Morin *et al.* (21) confirmed the mutation in HCT 116 and described a new mutation in SW 48, which we have confirmed. A homology search for functional motifs in β-catenin showed that the mutations detected appear to cause changes in consensus sequence sites and can potentially cause major changes in function.

The mutations in SW 48 (C-to-A change in codon 33) and HCT 116 (deletion of codon 45) result in loss of highly conserved serine residues in a region of the protein that may be a target for the enzyme GSK 3β. Both of these mutations are heterozygous, and Morin *et al.* (21) have demonstrated that these act in a dominant, negative fashion resulting in an increase in β-catenin-TCF 4-mediated transcriptional activity. In HCT 116, β-catenin coprecipitated with APC, E-cadherin, and α-catenin. Given the heterozygous nature of the mutation, it is impossible to know whether or not these complexes contained mutant protein. It does, however, show that in these cell lines, complex formation is not disrupted and that the major effects of these mutations are probably due to functional changes. In SW 48 the β-catenin protein coprecipitated with

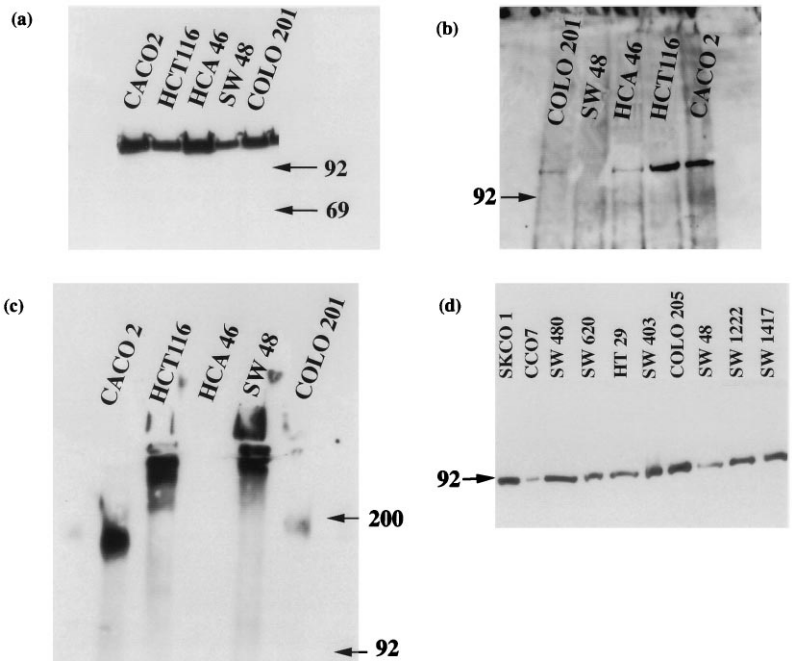

FIG. 2. (*a–c*) Coprecipitation of β-catenin in the cell lines with *CTNNB1* mutations. β-catenin was precipitated and blotted respectively with antibodies to (*a*) E-cadherin, (*b*) α-catenin, and (*c*) APC. All cell lines showed normal coprecipitation with E-cadherin (*a*). SW 48 failed to coprecipitate with α-catenin (*b*), and HCA 46 failed to coprecipitate with APC (*c*). In all other cases coprecipitation was observed as expected. In two of the cell lines, CCO7 and SW1417, there was no evidence of β-catenin mRNA by RT-PCR. Normal-sized protein was demonstrated by Western blot analysis (*d*), thus suggesting that there was loss of message stability in these cell lines.

E-cadherin and APC but not with α-catenin. Given that this is a heterozygous mutation, complexes of α-catenin with the wild-type β-catenin protein would be expected. There are many possibilities (such as gene silencing) to explain the absence of these complexes, but we do not yet know its basis.

The mutation in HCA 46 (G-to-T change in codon 183) causes a change from alanine to serine and is homozygous. This mutation introduces a new potential phosphorylation site into the protein, which results in a new consensus site for casein kinase 2 (CK-2), a serine/threonine kinase, which may be able to phosphorylate the newly introduced serine residue. This change may also alter the accessibility of the serine residue at position 179 (which occurs in a consensus site for CK-2) for phosphorylation through changes in tertiary structure. The mutation in HCA 46 is in the region of β-catenin, which complexes with APC. Our data show that there is no change in the ability to form complexes with E-cadherin or α-catenin, but there is no evidence of β-catenin/APC complexes. This is a heterozygous mutation, and complexes of APC would be expected with wild-type β-catenin. However, HCA 46, if it is expressing APC protein, does so at levels that are below the level of detection by Western blotting, and this would explain the absence of these complexes.

The mutation in $CACO_2$ (G-to-C change in codon 245) occurs in an amino acid that is directly N-terminal to a serine residue that lies in a consensus site for CK-2. The change in a −1 position may cause loss of target recognition by the protein kinase enzyme and would cause a potential change in the accessibility of that site for phosphorylation. The mutation occurs in the region responsible for APC binding but does not appear to affect the formation of β-catenin complexes with APC, E-cadherin, or α-catenin.

The cell lines Colo 201 and 205 are derived from the same patient, and both grow as semi-adherent cell lines. The floating component of each was examined separately to look for splice variants, which may explain the loss of adhesion. Both cell lines contain the same mutation (A-to-G change in codon 287), showing that the *CTNNB1* mutations occurred before metastasis. The mutation would result in a change from asparagine to serine, thereby introducing a new site for phosphorylation, although the mutated sequence does not appear to conform to any known consensus phosphorylation site. The mutation is in the region of β-catenin, which complexes with both APC and E-cadherin but does not appear to affect the formation of such complexes or those with α-catenin. This also suggests a functional change rather than one of complexing ability.

If one of the major effects of *APC* mutation is loss of control of normal β-catenin signaling, then, as argued by Morin *et al.*(21), in the presence of wild-type APC, *CTNNB1* mutations may give much the same effect. They found *CTNNB1* mutations in three of five tumors with wild-type APC. In our study there were four cell lines with wild-type APC (SW 48, HCT 116, HCA 7, and LS174T). Two of four of these cell lines had *CTNNB1* mutations, as opposed to 2 of 14 *CTNNB1* mutations in cell lines with *APC* mutations. This does not show a significant negative association between *CTNNB1* and *APC* mutation, although the trend is in the direction suggested by Morin *et al.* (21). The *APC* status of the cell line HCA 46 is as yet undetermined and is excluded from this analysis.

The cell lines $CACO_2$ and Colo 201/205 have both somatic *APC* mutations and *CTNNB1* mutations. The *CTNNB1* mutations in these cases must give a further selective advantage, perhaps boosting that due to escape from APC-mediated regulation. The same may be presumed true for the changes in mRNA levels in the cell lines CCO7 and SW 1417, both of which have *APC* mutations. Whether this subsequent advantage lies in changes in signaling or adhesion is as yet uncertain.

It is interesting that in three of the cases, *CTNNB1* mutations did not affect the ability of β-catenin to form complexes with other proteins. This may be explained by functional redundancy with γ-catenin (plakoglobin). This protein shares a high degree of homology with β-catenin and can form complexes with APC, α-catenin, and E-cadherin (22–25). Thus, mutations of β-catenin that alter complexing ability alone may be of little advantage if γ-catenin is able to take over the role of the mutated protein and thereby allow normal function. In one case, there was loss of complex formation with α-catenin (SW 48). In this case, a functional effect has already been described, and it is possible that failure to complex with α-catenin gives an additional selective advantage through disruption of the E-cadherin–catenin unit.

In summary, we have examined 23 human colorectal cancer cell lines for expressed mutations of *CTNNB1*. We found mutations in 26% of cases, and all mutations occurred in consensus sequences potentially causing functional modification of the protein. These results suggest that mutation of *CTNNB1*, causing functional alterations in adhesion and signaling, may be an important factor in the early development of colorectal cancers in the same general pathway as *APC* mutations.

1. Peifer, M., Berg, S. & Reynolds, A. B. (1994) *Cell* **76,** 789–791.
2. Peifer, M. (1995) *Trends Cell Biol.* **5,** 224–229.
3. McCrea, P. D., Turck, C. W. & Gumbiner, B. (1991) *Science* **254,** 1359–1361.
4. Gumbiner, B. M. & McCrea, P. D. (1993) *J. Cell. Sci. Suppl.* **17,** 155–158.
5. Gumbiner, B. M. (1995) *Curr. Opin. Cell Biol.* **7,** 634–640.
6. Peifer, M., McCrea, P. D., Green, K. J., Wieschaus, E. & Gumbiner, B. M. (1992) *J. Cell Biol.* **118,** 681–691.
7. Peifer, M., Orsulic, S., Sweeton, D. & Wieschaus, E. (1993) *Development* **118,** 1191–1207.
8. Funayama, N., Fagotto, F., McCrea, P. & Gumbiner, B. M. (1995) *J. Cell Biol.* **128,** 959–968.
9. McCrea, P. D., Brieher, W. M. & Gumbiner, B. M. (1993) *J. Cell Biol.* **123,** 477–484.
10. Wheelock, M. J. & Knudsen, K. A. (1991) *In Vivo* **5,** 505–513.
11. Brunner, E., Peter, O., Schweizer, L. & Basler, K. (1997) *Nature (London)* **385,** 829–833.
12. Behrens, J., von Kries, J., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, D. & Birchmeier, W. (1996) *Nature (London)* **382,** 638–642.
13. Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson, M. J., Godsave, S., Korinek, V., Roose, J., Destree, O. & Clevers, H. (1996) *Cell* **86,** 391–399.
14. Munemitsu, S., Albert, I., Souza, B., Rubinfeld, B. & Polakis, P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3046–3050.
15. Papkoff, J., Rubinfeld, B., Schryver, B. & Polakis, P. (1996) *Mol. Cell. Biol.* **16,** 2128–2134.
16. Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S. & Polakis, P. (1996) *Science* **272,** 1023–1026.
17. Inomata, M., Ochiai, A., Akimoto, S., Kitano, S. & Hirohashi, S. (1996) *Cancer Res.* **56,** 2213–2217.
18. Ilyas, M., Tomlinson, I., Rowan, A., Pignatelli, M. & Bodmer, W. (1997) *J. Pathol.* **181,** 36A (abstr.).
19. Yost, C., Torres, M., Miller, J. R., Huang, E., Kimelman, D. & Moon, R. T. (1996) *Genes Dev.* **10,** 1443–1454.
20. Ausubel, F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1994) *Current Protocols in Molecular Biology*, ed. Janssen, K. (Wiley, New York).
21. Morin, P., Sparks, A., Korinek, V., Barker, N., Clevers, H., Vogelstein, B. & Kinzler, K. (1997) *Science* **275,** 1787–1790.
22. Rubinfeld, B., Souza, B., Albert, I., Munemitsu, S. & Polakis, P. (1995) *J. Biol. Chem.* **270,** 5549–5555.
23. Butz, S., Stappert, J., Weissig, H. & Kemler, R. (1992) *Science* **257,** 1142–1144.
24. Knudsen, K. A. & Wheelock, M. J. (1992) *J. Cell Biol.* **118,** 671–679.
25. Ozawa, M., Baribault, H. & Kemler, R. (1989) *EMBO J.* **8,** 1711–1717.