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Expression of Wild-Type α-Catenin Protein in Cells with a Mutant α-Catenin Gene Restores Both Growth Regulation and Tumor Suppressor Activities

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Recent studies indicate that disruption of the E-cadherin-mediated cell-cell adhesion system is frequently associated with human cancers of epithelial origin. Reduced levels of both E-cadherin and the associated protein, α-catenin, have been reported in human tumors. This report describes the characterization of a human ovarian carcinoma-derived cell line (Ov2008) which expresses a novel mutant form of the α-catenin protein lacking the extreme N terminus of the wild-type protein. The altered form of α-catenin expressed in Ov2008 cells fails to bind efficiently to β-catenin and is localized in the cytoplasm. Deletion mapping has localized the β-catenin binding site on α-catenin between amino acids 46 and 149, which encompasses the same region of the protein that is deleted in the Ov2008 variant. Restoration of inducible expression of the wild-type α-catenin protein in these cells caused them to assume the morphology typical of an epithelial sheet and retarded their growth in vitro. Additionally, the induction of α-catenin expression in Ov2008 cells injected into nude mice attenuated the ability of these cells to form tumors. These observations support the classification of α-catenin as a growth-regulatory and candidate tumor suppressor gene.

The cadherins are a family of transmembrane glycoproteins which mediate Ca^{2+}-dependent, homotypic cell-cell adhesion in most solid tissues (35, 37). E-cadherin, the predominant cadherin expressed in epithelial cells, localizes to specialized membrane regions known as adherens junctions. Homophilic binding between cadherins on neighboring cells occurs through the direct interaction of their extracellular domains (14). The highly conserved cytoplasmic domain of E-cadherin is associated with a group of proteins collectively known as catenins (α-, β- and γ-catenin) (19, 24) which have been shown to be essential for cadherin function (8, 18, 25). Either β-catenin or γ-catenin (plakoglobin) interacts directly with E-cadherin in discrete complexes (4), while α-catenin, which does not bind to E-cadherin directly, is subsequently recruited to the complex through its interaction with either β- or γ-catenin (1, 6). α-Catenin, which has sequence similarity to vinculin, also contacts actin filaments and consequently serves as a connector between intercellular adhesion and the actin-based cytoskeleton (3, 20, 25). A fourth cadherin-associated protein, $p120^{cas}$, (28) has recently been found associated with cadherin complexes; although it is suggested to play a regulatory role in the assembly of adherens junctions, its precise function remains to be elucidated.

A number of recent studies suggest that disruption of the cadherin-based adhesion system may play a role in the progression of human tumors. Immunohistochemical staining has revealed reduced levels of both E-cadherin and α-catenin proteins in a variety of human tumors, including tumors of the breast, colon, stomach, esophagus, bladder, and liver (7, 11, 15, 16, 21, 29, 31, 32), while higher levels of expression are maintained in the normal epithelium of these tissues. The portion of tumors that fail to express one or both of these proteins has been reported to be as high as 80% of those examined, and a correlation exists between the loss of E-cadherin or α-catenin and the degree of lymph node metastasis and tumor dedifferentiation (7, 15, 30, 38). The loss of α-catenin appears to be the more common event and also correlates somewhat better than loss of E-cadherin with the level of invasiveness of a tumor (7, 15, 33). Recently, the reduction of β-catenin expression in a variety of human tumors has also documented (34).

The present study describes a cell line which harbors a point mutation within the α-catenin gene resulting in the production of an N-terminally deleted protein which fails to bind to β-catenin. E-cadherin-mediated cell-cell adhesion was restored in this cell line by introducing the wild-type α-catenin gene under the control of an inducible promoter. The expression of the wild-type α-catenin gene product (i) restored normal epithelial cell morphology, (ii) retarded the growth of this cell line in culture, and (iii) reduced the ability of these cells to grow as tumors in nude mice. These data demonstrate that α-catenin can act as a growth-regulatory and tumor suppressor gene.

MATERIALS AND METHODS

Cell culture and DNA transfections. The human breast epithelial cell line T47D was obtained from Bert Vogelstein (Johns Hopkins School of Medicine), and the human ovarian carcinoma cell line Ov2008 was obtained from Dennis Slamon (UCLA School of Medicine). 293 (a kidney epithelial cell line) and the lung epithelial cell line H1299 were laboratory stock cell lines. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum in a humidified 5% CO_2 atmosphere at 37°C.

All transfections were performed by using the Lipofectamine reagent (GIBCO/BRL) as directed by the manufacturer. Transient transfections were assayed 48 h posttransfection. To establish stable cell lines, Ov2008 cell clones were selected in hygromycin B (150 U/ml; Calbiochem) 72 h following transfection with plasmid pGREαcat (described below). Isolated clones were expanded and screened for the inducible expression of α-catenin protein by Western blotting.

Ov2008 cells were used for growth suppression assays. Cells were grown to approximately 80% confluency in 10-cm-diameter tissue culture dishes and were then transfected with 5 μg of plasmid DNA (either pGRE5-1/EBV or pGREαcat). At 48 h posttransfection, the cells were collected by trypsinization and seeded in 10-cm-diameter dishes in duplicate at an initial density of 10^5 cells. Twenty-four hours after plating, the medium was changed to include hygromycin B at 150 U/ml. At this point, dexamethasone (Sigma) was added to half of the plates at a concentration of 500 nM. The cells were maintained at 37°C, and the medium was changed every 4 days. Approximately 4 weeks after plating, colonies were stained with crystal violet.

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Antibodies. The E-cadherin monoclonal antibody was purchased from Transduction Laboratories. The β-catenin monoclonal antibody (2E1) and all of the α-catenin monoclonal antibodies used in this study were generated in our laboratory as follows. BALB/c mice were immunized with a glutathione *S*-transferase fusion of the full-length human α- or β-catenin protein purified from *Escherichia coli* (a gift from David Rimm, Yale University). Hybridomas were prepared by using standard procedures and were subsequently screened by using an enzyme-linked immunosorbent assay. Stable clones were established by three rounds of cloning, and low-resolution epitope mapping was carried out for each of the α-catenin monoclonal antibodies based on their ability to immunoprecipitate each of the α-catenin deletion mutants described in this report. In this study, we generated eight monoclonal antibodies which recognize epitopes within the regions bounded by the amino acids indicated in parentheses: 3H4 (84 to 129), 2G12, 3C1, 6A1, and 6A9 (129 to 228), 5B11 and 7A11 (228 to 326), and 10E1 (326 to 907). It has not yet been determined whether the antibodies with epitopes which map to the same region recognize the same or unique epitopes.

Plasmids and cloning. Plasmid pCGN (36) is an expression vector which expresses foreign genes as N-terminal influenza virus hemagglutinin (flu) epitope-tagged fusion proteins. Plasmid pGRE5-1/EBV (13), which was obtained from United States Biochemical Corp., is a eukaryotic episomal expression vector which confers resistance to hygromycin. Inducible expression of foreign genes is directed by the adenovirus major late promoter and five upstream glucocorticoid response elements. Plasmid pαcat1 contains the full-length human α-catenin cDNA (22), which was obtained from Setsuo Hirohashi (National Cancer Center Research Institute, Tokyo, Japan), cloned into the *Eco*RI site of pBluescript KS (Stratagene).

The full-length clone of human α-catenin cDNA was amplified by using Advantage KlenTaq (Clontech) and PCR from the plasmid pαcat1, using the forward primer AcatS (5′-CGCGGTACCAAATGACTGCTGTCCATG-3′) and the reverse primer AcatR (5′-CGCGGTACCTGGGCAGACTTAGATG-3′), which incorporate *Kpn*I sites on the ends. The 2.7-kb fragment generated in this manner was digested with *Kpn*I, purified, and ligated into the *Kpn*I site of vector pGRE5-1/EBV to generate plasmid pGREαcat. This same PCR fragment was ligated into the *Kpn*I site of vector pCGN downstream of and in frame with the flu epitope tag to generate the full-length construct 1-907. To generate N-terminal α-catenin deletion mutants, pαcat1 was first digested with *Eco*RV to generate a blunt 3′ end. Digestion with the restriction enzyme indicated in parentheses following each mutant was then performed to delete upstream coding sequences: 46-907 (*Apa*I), 129-907 (*Xho*I), and 227-907 (*Sph*I). These fragments were purified, treated with Klenow enzyme to generate a blunt 5′ end, and ligated to vector pCGN, which had been digested with *Sma*I. The remaining mutants harboring N-terminal deletions were generated by PCR from plasmid pαcat1, using AcatR as the reverse primer and the following forward primers: 23-907 (5′-CGCGGTACCTGGCAGTTGAGAGACTG-3′), 84-907 (5′-CGCGGTACCAGTTTCTCAAGGAGGAGC-3′), and 100-907 (5′-CGCGGTACCAAGGTGATTTGATGAAGGC-3′). The mutants containing C-terminal deletions were all generated by using AcatS as a forward primer and the following reverse primers: 1-111 (5′-CGCGGTACCCTACTCTCCTGCAGCAGCCTT-3′), 1-130 (5′-CGCGGTACCCTAAGCTCGAGCTGCCCGAAC-3′), 1-138 (5′-CGCGGTACCCTACCGGGTAACAGCAGAGAG-3′), 1-149 (5′-CGCGGTACCCTATTTGTAGACATCTGCCATGTC-3′), 1-161 (5′-CGCGGTACCCTAGATACCATCTTCCACAAC-3′), 1-198 (5′-CGCGGTACCCTATTCCTGTTGTCTTTTGGCTG-3′), and 1-225 (5′-CGCGGTACCCTATGCAGTATAGAGGATCGG-3′). PCR products were digested with *Kpn*I and ligated into the *Kpn*I site of pCGN. All of the deletion mutants described above were cloned in frame with the flu epitope tag to generate fusion proteins.

Immunoprecipitations and Western blotting. For analysis of cadherin-catenin complexes, Ov2008 or T47D cells were metabolically labeled for several hours in methionine-free medium containing [^{35}S]methionine at 50 μCi/ml. Protein extracts were prepared by sonication in lysis buffer (50 mM Tris-Cl [pH 8.0], 5 mM EDTA, 150 mM NaCl, 0.5% Nonidet P-40) containing the following protease inhibitors: 1 mM phenylmethylsulfonyl fluoride, 0.2 μM aprotinin, and 1 μM E-64. Then 10^6 cpm of each extract was incubated with 5 mg of protein A-Sepharose 4B (Sigma) and monoclonal antibody against E-cadherin, α-catenin (5B11), or β-catenin (2E1) for 6 h at 4°C. Immunoprecipitates were washed three times with SNNTE (5% sucrose, 50 mM Tris-Cl [pH 7.4], 5 mM EDTA, 0.5 M NaCl, 1% Nonidet P-40) and then once with radioimmunoprecipitation assay buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulfate [SDS], 1% sodium deoxycholate), and bound proteins were boiled in sample buffer (10% glycerol, 2% SDS, 0.02% bromophenol blue, 10% β-mercaptoethanol, 125 mM Tris-Cl [pH 6.8]) prior to fractionation on an SDS–8% polyacrylamide gel. Gels were fixed, soaked in a solution of 1 M sodium salicylate, dried, and exposed to film. For the β-catenin coimmunoprecipitation assays, 293 cells were transiently transfected with plasmids encoding each of the α-catenin deletion mutants. Each extract (150 μg) was subject to immunoprecipitation with the anti-flu epitope tag monoclonal antibody 12CA5, and immunoprecipitates were washed with SNNTE. Following electrophoresis, proteins were electroblotted to nitrocellulose and subjected to Western analysis using either the anti-α-catenin or the anti-flu epitope monoclonal antibody to confirm expression of the α-catenin fragments and anti-β-catenin monoclonal antibody 2E1 to assay for coimmunoprecipitation of β-catenin. Following incubation with horseradish peroxidase-conjugated anti-mouse immunoglobulin G (Transduction Laboratories), immunocomplexes were visualized by using the Amersham ECL (enhanced chemiluminescence) detection system.

Reverse transcriptase PCR. Total RNA was prepared from Ov2008 cells by the TriZol (GIBCO/BRL) method, and DNA contaminants were removed by using a Message Clean kit (GenHunter). Total RNA (5 μg) was primed with random hexamers (Pharmacia) and reverse transcribed by using Moloney murine lymphotrophic virus Superscript reverse transcriptase (GIBCO/BRL) as described previously (5). PCR was performed by using Hot Tub polymerase (Amersham) with primers ACAT-1 (5′-ATGACTGCTGTCCATGCAGGCAAC-3′), which binds to the α-catenin cDNA starting with the first ATG, and ACAT-2 (5′-TGAGGCATCGTCTGAGGCAGTGGC-3′), which binds from nucleotides 870 to 846. PCR was performed for 35 cycles, each consisting of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. The 870-bp fragment generated in this manner was purified and ligated directly into the TA cloning vector (InVitrogen) and sequenced completely, using a Sequenase kit (United States Biochemical).

Immunofluorescence. Cells were cultured on glass coverslips prior to fixation at −20°C with 50% methanol–50% acetone. After several washes in phosphate-buffered saline, the cells were incubated with either α-catenin monoclonal antibody 3C1, β-catenin monoclonal antibody 2E1, or the E-cadherin monoclonal antibody. This was followed by sequential incubation with biotinylated goat anti-mouse immunoglobulin G and Cy-3-conjugated streptavidin (both from Jackson Immunoresearch Laboratories). Images were obtained with a confocal microscope (Bio-Rad model MRC600).

Tumor formation. To assay for tumorigenicity, Ov2008 or Ov2008(α1) cells were injected subcutaneously into opposite flanks of BALB/c *nu*/*nu* mice; 5×10^6 cells were injected, and tumor growth was monitored daily. When tumors had reached a diameter of 3 to 4 mm, 10 μg of dexamethasone phosphate (Elkins-Sinn, Inc.) was injected daily into the hind legs of the animals.

RESULTS

Identification of a cell line expressing a novel mutant form of α-catenin. To identify alterations in the E-cadherin–catenin cell adhesion system in cultured cell lines, protein extracts were prepared from a collection of epithelial cell lines derived from human tumors and screened by Western blotting with monoclonal antibodies against E-cadherin and α- or β-catenin. In the course of this screen, we identified an ovarian carcinoma cell line, designated Ov2008, in which the full-length, 102-kDa α-catenin protein was not detected. However, in this cell line, the α-catenin monoclonal antibodies reacted with a protein of slightly lower apparent molecular mass (approximately 90 kDa) which was not observed in other cell lines in this screen. To determine if this smaller protein was a truncated form of α-catenin, radiolabeled extracts were prepared from Ov2008 cells and immunoprecipitation reactions were carried out with each of a panel of anti-α-catenin monoclonal antibodies which are described in Materials and Methods. The 90-kDa protein was immunoprecipitated efficiently by seven of the eight monoclonal antibodies tested (data not shown) and therefore was considered likely to represent an altered form of α-catenin and not a cross-reacting protein.

When immunoprecipitations were performed with extracts from a control cell line (T47D), which expresses the wild-type α-catenin protein, the entire cadherin-catenin complex (including E-cadherin, either β- or γ-catenin, and α-catenin) was coimmunoprecipitated by antibodies directed against any of these components (Fig. 1). However, when the α-catenin variant was immunoprecipitated from Ov2008 cells, neither E-cadherin nor β-catenin was coimmunoprecipitated, although both of these proteins were present and complexed with each other (Fig. 1). It was noted that one of the anti-α-catenin monoclonal antibodies, 3H4, immunoprecipitated little or none of the truncated α-catenin protein from Ov2008 cells although this antibody efficiently immunoprecipitates α-catenin from T47D cells (data not shown). Furthermore, much lower levels of both β-catenin and E-cadherin were coimmunoprecipitated with α-catenin from T47D cell extracts by using the 3H4 antibody compared to each of the other anti-α-catenin monoclonal antibodies (data not shown).

Epitope mapping indicated that the 3H4 monoclonal anti-

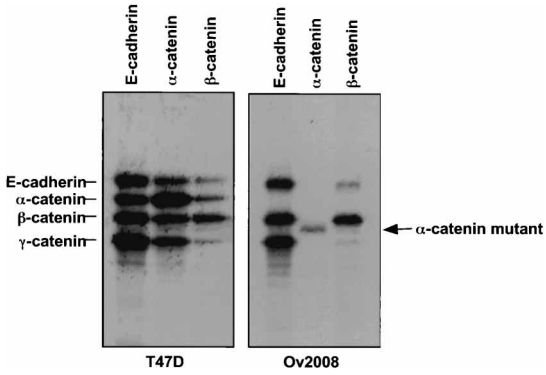

FIG. 1. Immunoprecipitation analysis of the cadherin-catenin complexes from Ov2008 and T47D cell lines. Cells were metabolically labeled, and cell extracts were subject to immunoprecipitation with monoclonal antibodies directed against the protein indicated at the top of each lane. The positions of migration of E-cadherin (120 kDa) and α (102 kDa)- β (92 kDa)-, and γ (82 kDa)-catenins are indicated to the left, and the position of the mutant form of α-catenin in Ov2008 cells is indicated to the right.

body recognizes an epitope in the extreme N terminus of the α-catenin protein, between amino acids 84 and 129, thereby suggesting that this region is altered or deleted from the α-catenin variant expressed in Ov2008 cells. The observation that this variant does not associate with the cadherin-catenin complex suggests that this deleted region may contain the β-catenin binding site. Furthermore, these data suggest that the epitope recognized by the 3H4 monoclonal antibody may overlap the β-catenin binding site and therefore compete with β-catenin for binding to α-catenin.

Determination of the α-catenin mutation in the Ov2008 cell line. The immunoprecipitation results suggested that the defect in α-catenin in Ov2008 cells involved the deletion of a region in the N terminus of the protein. To determine the nature of the corresponding mutation in the gene, the 5′ region of the α-catenin cDNA from Ov2008 cells was cloned by using reverse transcriptase PCR. Primers were selected to amplify an 870-nucleotide region from the 5′ end of the wild-type cDNA, beginning with the initiating ATG codon, from both Ov2008 cells and the control cell line T47D. An 870-bp fragment was the predominant product amplified from both cell lines, indicating that the defect in Ov2008 cells was not likely to result from a deletion or aberrant splicing within this region. Sequencing identified a single point mutation in the cDNA derived from Ov2008 cells, a C-to-T transition at nucleotide position 103 (Fig. 2), which was confirmed in six cDNA clones generated from two independent reverse transcriptase reactions. This mutation results in the replacement of the wild-type glutamine (CAG) codon at amino acid 35 with an amber stop (TAG) codon, thus terminating translation prematurely.

Since there is no deletion within the cDNA sequence and the protein product is missing an epitope located at the N terminus, it seems likely that translation of the mutant protein results from reinitiation at an internal methionine residue. As seen in Fig. 2, the next AUG codon encountered in the α-catenin sequence corresponds to amino acid 104. Initiation at this AUG codon would produce a protein with a predicted molecular mass of 89 kDa, which is consistent with the molecular mass of the α-catenin variant in Ov2008 cells as estimated from its electrophoretic mobility. The methionine at amino acid residue 104 is therefore the most likely candidate for the translational start site of the α-catenin protein in Ov2008 cells.

Mapping the β-catenin binding domain on α-catenin. The β-catenin binding domain has previously been broadly assigned to a region in the N-terminal 660 (of 907) amino acids of the α-catenin protein (6) but has not been more precisely mapped. Analysis of the α-catenin mutant from Ov2008 cells suggested that a region within the N-terminal 104 amino acids of α-catenin is required for β-catenin binding. To determine whether this region is sufficient for binding and to more precisely map the binding site, α-catenin deletion mutants were constructed in this region and used to assay β-catenin binding. Mutants were constructed as described in Materials and Methods by subcloning regions of the gene into an expression vector which expresses the fragments as N-terminal fusions with the flu epitope tag. The panel of α-catenin deletion mutants were expressed in 293 cells following transient transfection and subsequently immunoprecipitated with a monoclonal antibody directed against the flu epitope tag. Stable expression of each protein fragment was confirmed by Western blotting with either anti-α-catenin or anti-flu epitope tag antibodies, and coimmunoprecipitation of β-catenin was assayed by Western blot analysis using the anti-β-catenin monoclonal antibody. Fourteen different α-catenin constructs were tested in this assay,

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1    ATG ACT GCT GTC CAT GCA GGC AAC ATA AAC TTC AAG TGG GAT CCT AAA AGT CTA GAG ATC  60
1    Met thr ala val his ala gly asn ile asn phe lys trp asp pro lys ser leu glu ile  20
                                                          TAG
61   AGG ACT CTG GCA GTT GAG AGA CTG TTG GAG CCT CTT GTT ACA CAG GTT ACA ACC CTT GTA  120
21   arg thr leu ala val glu arg leu leu glu pro leu val thr gln val thr thr leu val  40
                                                          AMB
121  AAC ACC AAT AGT AAA GGG CCC TCT AAT AAG AAG AGA GGT CGT TCT AAG AAG GCC CAT GTT  180
41   asn thr asn ser lys gly pro ser asn lys lys arg gly arg ser lys lys ala his val  60

181  TTG GCT GCA TCT GTT GAA CAA GCA ACT GAG AAT TTC TTG GAG AAG GGG GAT AAA ATT GCG  240
61   leu ala ala ser val glu gln ala thr glu asn phe leu glu lys gly asp lys ile ala  80

241  AAG GAG AGC CAG TTT CTC AAG GAG GAG CTT GTG GCT GCT GTA GAA GAT GTT CGA AAA CAA  300
81   lys glu ser gln phe leu lys glu glu leu val ala ala val glu asp val arg lys gln  100

301  GGT GAT TTG ATG AAG GCT GCT GCA GGA GAG TTC GCA GAT GAT CCC TGC TCT TCT GTG AAG  360
101  gly asp leu met lys ala ala ala gly glu phe ala asp asp pro cys ser ser val lys  120

361  CGA GGC AAC ATG GTT CGG GCA GCT CGA GCT TTG CTC TCT GCT GTT ACC CGG TTG CTG ATT  420
121  arg gly asn met val arg ala ala arg ala leu leu ser ala val thr arg leu leu ile  140

421  TTG GCT GAC ATG GCA GAT GTC TAC AAA TTA CTT GTT CAG CTG AAA GTT GTG GAA GAT GGT  480
141  leu ala asp met ala asp val tyr lys leu leu val gln leu lys val val glu asp gly  160

481  ATC TTG AAG TTG AGG AAT GCT GGC AAT GAA CAA GAC TTA GGA ATC CAG TAT AAA GCC CTA  540
161  ile leu lys leu arg asn ala gly asn glu gln asp leu gly ile gln tyr lys ala leu  180
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FIG. 2. Nucleotide and predicted amino acid sequences corresponding to the first 180 amino acids of the wild-type human α-catenin protein. The nucleotide sequence is numbered with the first ATG codon assigned position 1. The location and consequence of the mutation detected in the α-catenin variant expressed in Ov2008 cells are indicated in the boxed region, and the locations of the three downstream methionine residues which occur within this sequence are indicated with shading.

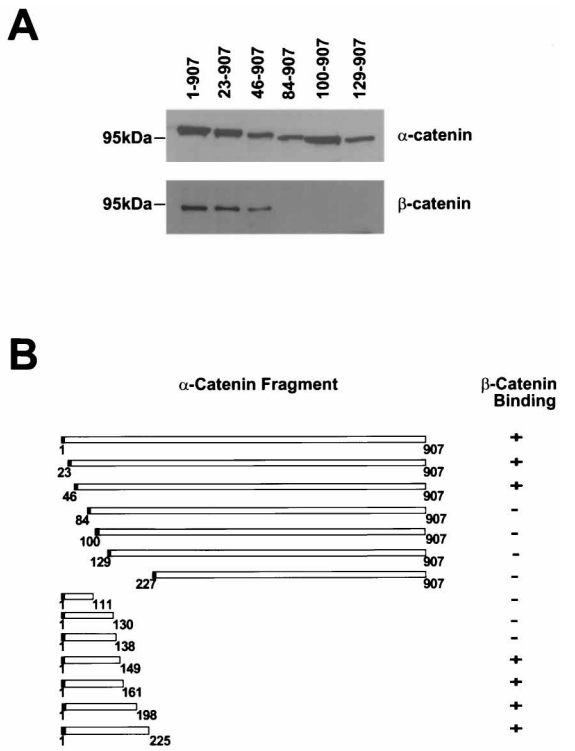

FIG. 3. β-Catenin binding activity of α-catenin deletion mutants. Fourteen α-catenin deletion mutants were constructed in this study and tested for binding to β-catenin. (A) Representative data for the N-terminal deletion mutants. 293 cells were transiently transfected with plasmids encoding each of these mutants followed by immunoprecipitation of each fragment with an antibody directed against the flu epitope tag. Western blot analysis was carried out with anti-α-catenin antibodies (upper panel) to confirm expression of the α-catenin mutants and anti-β-catenin antibodies (lower panel) to assay for β-catenin binding. (B) Summary of results for all of the mutants tested. The portion of the α-catenin protein encoded by each mutant is represented by an open bar. The shaded region represents the N-terminal flu epitope tag, and the numbers indicate the amino acids forming the boundaries of each mutant. The ability of each mutant to coimmunoprecipitate β-catenin in this assay is indicated at the right.

and the results are summarized in Fig. 3. Deletion of the N-terminal region between amino acids 46 and 84 abolished β-catenin binding. A fragment containing only the N-terminal 149 amino acids bound to β-catenin in this assay, while a slightly smaller fragment containing only the first 138 amino acids did not. These results support the assignment of the β-catenin binding site to the N terminus of the protein and map the binding site within the region bounded by amino acids 46 and 149. The proposed deletion of 104 amino acids from the N terminus of the α-catenin variant observed in Ov2008 cells and the 3H4 monoclonal antibody epitope overlap this region.

Characterization of an Ov2008-derived cell line with inducible wild-type α-catenin protein expression. Initial efforts to isolate an Ov2008-derived cell line which constitutively overexpressed wild-type α-catenin were unsuccessful. Following transfection, drug-resistant colonies formed initially but the majority ceased to proliferate and could not be expanded. Although immunofluorescent staining of the early colonies revealed α-catenin protein localized to the cell membrane, Western blot analysis of the few clones that continued to divide revealed that they were no longer expressing the wild-type protein. These results suggest that expression of high levels of α-catenin is inhibitory to cell growth. Therefore, to restore wild-type α-catenin expression in this cell line, it was necessary to introduce the gene under the control of an inducible promoter. We chose an expression vector in which transcription is regulated by five glucocorticoid response elements and gene expression is induced by the addition of a corticosteroid such as dexamethasone to the tissue culture medium (13).

The full-length α-catenin cDNA was cloned into the vector pGRE5-1/EBV, and the resulting plasmid was introduced into Ov2008 cells by transfection. Individual colonies were selected and propagated in the presence of hygromycin B, and a stable cell line, designated Ov2008(α1), was obtained. Western blot analysis followed by densitometry indicated that in the presence of dexamethasone, the level of expression of wild-type α-catenin in Ov2008(α1) cells is equivalent to the level of α-catenin in T47D cells. However, there is leaky expression of α-catenin in the absence of dexamethasone, which results in levels of wild-type α-catenin that are 28% of those seen in T47D. We also isolated a second cell line, Ov2008(α2), in which the wild-type protein was undetectable in the absence of dexamethasone and which upon induction expressed levels of α-catenin that were 42% of those observed in Ov2008(α1) cells. Similar phenotypic alterations were observed in both Ov2008(α1) and Ov2008(α2) cells in response to dexamethasone. However, the changes were more dramatic in Ov2008(α1) cells, presumably due to the higher levels of α-catenin protein induced, and therefore the results obtained with this cell line are presented in this study.

Immunofluorescent staining was used to determine the cellular localization of E-cadherin and the catenins in Ov2008 cells (Fig. 4). The control cell line T47D shows the membrane association of α- and β-catenin and E-cadherin characteristic of epithelial cells. In Ov2008 cells, both E-cadherin and β-catenin were localized at the cell membrane, but the endogenous mutant α-catenin had a diffuse cytoplasmic distribution. Immunofluorescent staining for α-catenin in the Ov2008(α1) cell line (Fig. 5) demonstrated that significant levels of α-catenin localized to the membrane only in the induced state. In the absence of dexamethasone, the majority of the α-catenin expressed was localized in the cytoplasm, although a small amount was detectable in association with the cell membrane. In addition, immunoprecipitation analysis of complexes containing E-cadherin in Ov2008(α1) cells revealed that a significant amount of wild-type α-catenin protein was coimmunoprecipitated with E-cadherin antibodies only in the induced state (data not shown).

Uninduced Ov2008(α1) cells have a morphology similar to that of the parental Ov2008 cells. The cells appear scattered and disorganized. However, when dexamethasone was added to the culture media, the Ov2008(α1) cells underwent a rapid and dramatic morphological transformation whereas the Ov2008 cells were unaffected (Fig. 6). Within several hours of the induction of α-catenin expression, the Ov2008(α1) cells appeared larger and flatter and acquired the extensive cell-cell contacts and organized "cobblestone" appearance typical of epithelial cells. Taken together, these observations suggested that the lower levels of full-length α-catenin expressed in Ov2008(α1) cells in the absence of inducer were not sufficient to alter the cell morphology or to effectively form complexes with E-cadherin and β-catenin. However, when dexamethasone is added to the medium, productive cadherin-catenin complexes form, making this cell line a useful tool for studying the biological effects of α-catenin expression.

Effect of α-catenin expression on the growth of Ov2008(α1) cells. To assess the effect of wild-type α-catenin expression on the proliferative rate of Ov2008 cells in culture, growth rates were determined for the Ov2008(α1) cell line with and without the induction of α-catenin expression (Fig. 7). When dexamethasone was included in the culture medium, Ov2008(α1)

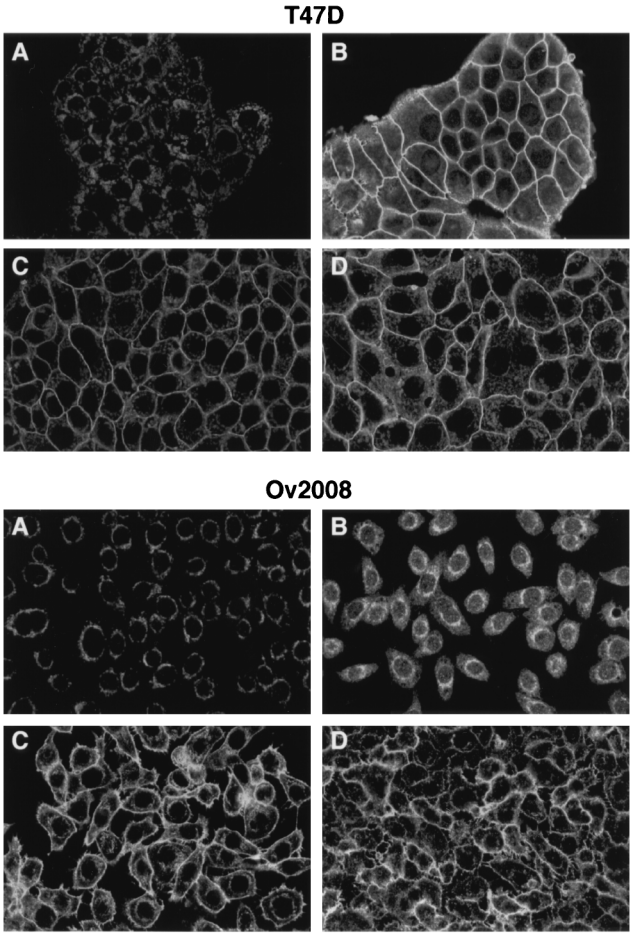

FIG. 4. Immunofluorescent staining for E-cadherin and α- and β-catenin. T47D or Ov2008 cells were plated on coverslips and incubated with the indicated monoclonal antibodies followed by immunofluorescent detection of bound antibody. Negative controls (A) were incubated without primary antibody. (B) E-cadherin; (C) α-catenin; (D) β-catenin.

cells exhibited significantly slower growth than the parental Ov2008 line. In the absence of dexamethasone, Ov2008(α1) cells had an intermediate growth rate which is likely to be attributable to the lower levels of wild-type α-catenin expressed in these cells even in the uninduced state. Growth of the Ov2008(α2) cell line was also retarded by the induction of α-catenin expression, and the degree of repression was consistent with the lower levels of α-catenin expression induced in this cell line (data not shown). The growth of Ov2008 cells was unaffected by the presence of dexamethasone. These data suggest that α-catenin has an inhibitory effect on cell growth and that the degree of this inhibition correlates with the level of α-catenin protein expressed.

Suppression of colony formation by α-catenin. A tissue culture assay was established to further test the contention that α-catenin exerts a negative effect on cell growth. Ov2008 cells were transfected with pGREαcat and selected for resistance to hygromycin B in the presence or absence of dexamethasone. As can be seen in Fig. 8, there is no difference in the number of drug-resistant colonies formed following transfection with pGREαcat or the pGRE vector control in the absence of dexamethasone. However, when α-catenin expression is induced by the addition of dexamethasone, the number of colonies formed by pGREαcat is dramatically reduced compared to the vector control. Quantification of this difference by counting the colonies reveals an approximately 90-fold suppression of colony formation by the overexpression of full-length α-catenin.

To demonstrate specificity of this growth suppression, several of the α-catenin deletion mutants were subcloned into the pGRE vector and assayed for the ability to suppress colony formation. Some, but not all, of the constructs inhibit the formation of colonies upon induction with dexamethasone (data not shown). To demonstrate that the observed growth suppression is specific to cells deficient in α-catenin and is not a consequence of the overexpression of α-catenin, colony formation assays were also performed in three epithelial cell lines which express wild-type α-catenin. T47D, H1299, and 293 cells were transfected with plasmid pGREαcat and treated as described above. There was no suppression of colony formation upon induction of α-catenin expression in any of these cell lines (data not shown).

Effect of α-catenin expression on the tumorigenicity of Ov2008(α1) cells. To determine whether α-catenin suppresses growth in vivo as well as in vitro, the ability of Ov2008 and Ov2008(α1) cells to form tumors in nude mice was evaluated. Mice were injected with 5×10^6 cells of the parental cell line (Ov2008) and the α-catenin-inducible cell line Ov2008(α1) on opposite flanks, and tumor growth was monitored. Tumors first became apparent on both flanks in 4 to 6 days and grew rapidly. In a control group of mice, the tumors derived from both cell lines continued to grow at similar rates, reaching an average size of 7.8 mm by 16 days. These data suggest that the lower level of α-catenin expressed in Ov2008(α1) cells in the absence of dexamethasone was not sufficient to inhibit tumor growth. In a second group of mice, daily intramuscular injection of dexamethasone phosphate was begun once the tumors

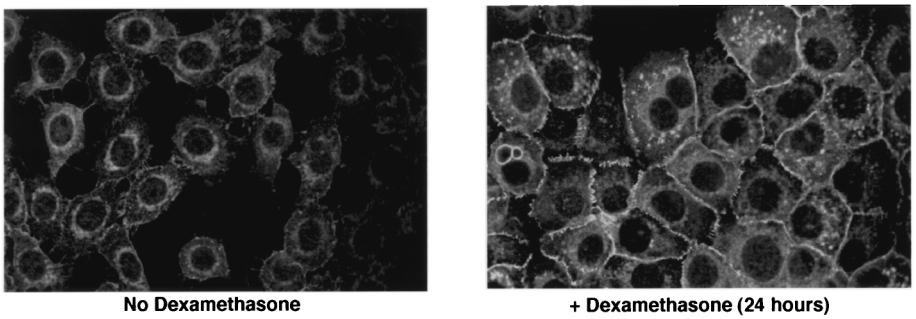

FIG. 5. Induction of α-catenin expression in Ov2008(α1) cells. α-Catenin expression was induced in Ov2008(α1) cells by the addition of 500 nM dexamethasone to the culture medium. The α-catenin protein was visualized 24 h later by immunofluorescent staining.

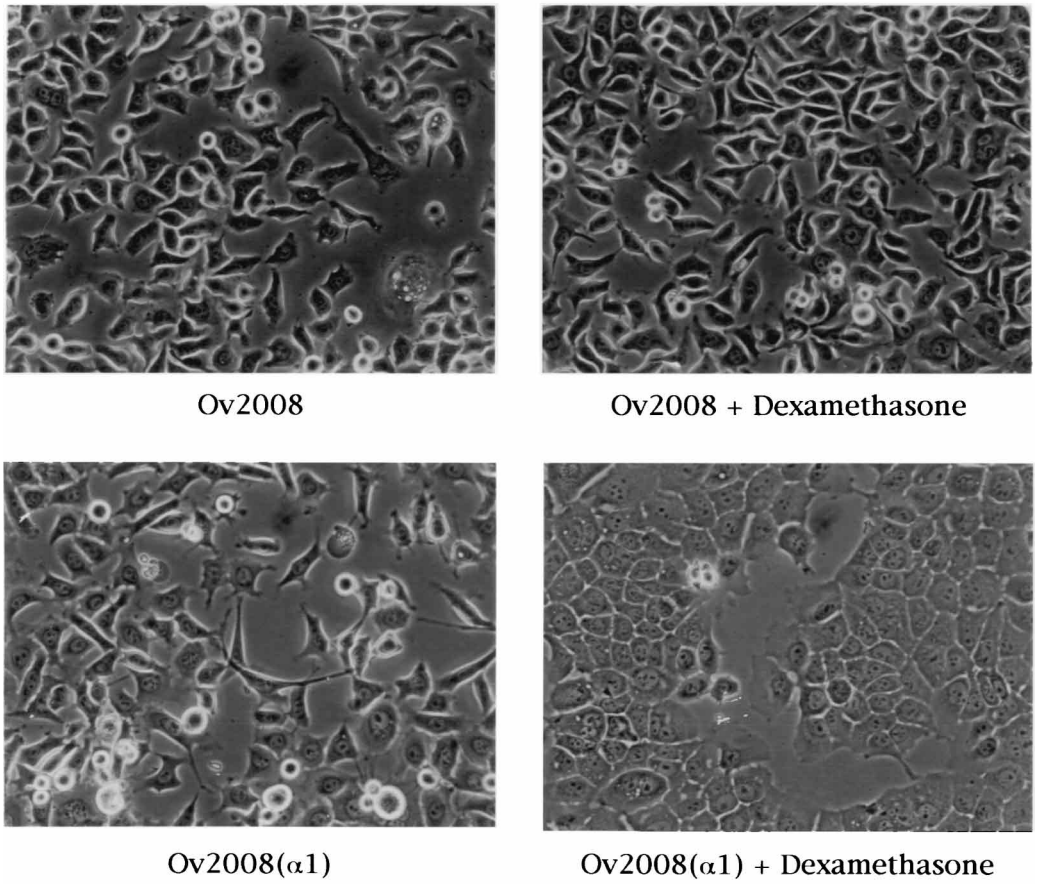

FIG. 6. Morphological changes in Ov2008 and Ov2008(α1) cells upon addition of dexamethasone. Ov2008 and Ov2008(α1) cells were grown in the presence or absence of 500 nM dexamethasone. Pictures were taken with a phase-contrast microscope 24 h after the addition of dexamethasone, although changes in cell morphology became apparent as soon as 4 h after induction.

on both flanks had reached a diameter of 3 to 4 mm (7 days after tumor cell injection). As indicated in Fig. 9, the administration of dexamethasone to induce wild-type α-catenin expression in the Ov2008(α1) cells was associated with inhibition of the growth of the Ov2008(α1) tumors, while the Ov2008 tumors continued to grow rapidly. From days 7 to 15, the Ov2008 tumors grew by approximately 5.8 mm whereas Ov2008(α1) tumors grew by only 1.5 mm. These observations support the conclusion that α-catenin expression inhibits the growth of these tumors in vivo. In a third experimental group, daily dexamethasone injections were initiated at the time of tumor cell injection. The emergence of Ov2008(α1) tumors was slightly delayed in this group, but they did eventually form (data not shown). Although the administration of dexamethasone did not prevent tumor formation, it is possible that the cells which formed tumors in these animals had eliminated α-catenin expression in response to selective pressure. This is currently being investigated.

DISCUSSION

Disruption of the cadherin-catenin intercellular adhesion system has been implicated in the progression of human cancers. The α-catenin protein is frequently undetectable in tumor tissue, and several tumor-derived cell lines which do not express the α-catenin protein have also been described (17, 22, 27, 38). To date, the defect has been defined in two of these cell lines, and both lack α-catenin expression due to the deletion of portions of the α-catenin gene (17, 22). Here, we describe a human ovarian carcinoma-derived cell line (Ov2008) in which a mutant form of the α-catenin protein is expressed. Sequence analysis of α-catenin cDNAs isolated from this cell line identified a base substitution mutation which introduces a stop codon 103 nucleotides downstream of the first AUG codon. The mutant protein that we have detected in this cell line likely results from the reinitiation of translation from the next in-frame AUG codon, which would result in the deletion of 104 amino acids from the N terminus of the protein. Several lines of evidence support this model: (i) a monoclonal antibody which recognizes an epitope in the extreme N terminus of α-catenin fails to bind the truncated form α-catenin; (ii) the mutant α-catenin does not bind to β-catenin, and we have mapped the β-catenin binding site to a region in the N terminus of the protein; and (iii) α-catenin monoclonal antibodies detect a protein of 90 kDa in this cell line, which is consistent with the predicted molecular mass of a protein initiating at the second methionine residue. Although according to the scanning model, eukaryotic translation generally begins at the most 5′ AUG codon within the mRNA (10), experimental evidence supports the notion that ribosomes are capable of efficiently reinitiating at the next AUG codon when an in-frame translation termination codon is introduced downstream of the 5′ initiation codon (9, 12, 26).

In Ov2008 cells, E-cadherin and β-catenin were found com-

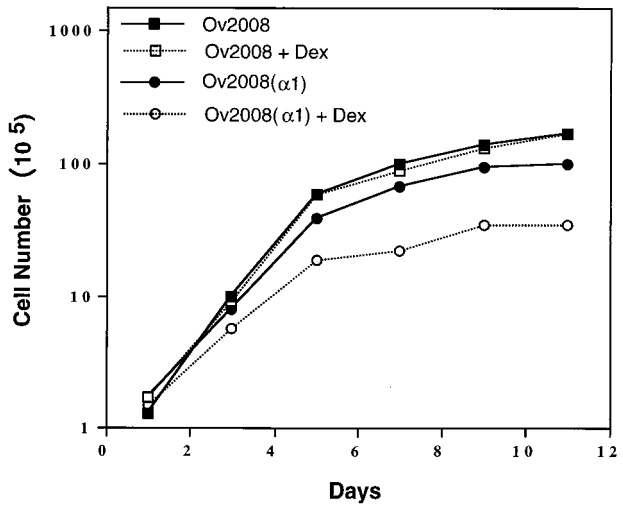

FIG. 7. Growth rates of Ov2008 and Ov2008(α1) cells in the presence or absence of dexamethasone (Dex). Cells were seeded at an initial density of 10^5 cells per 10-cm-diameter dish with or without 500 nM dexamethasone. Cells were collected by trypsinization and counted in a hemacytometer over the course of 16 days to determine the in vitro growth of the cell lines. Cells from two plates were counted at each time point and averaged to generate the graph.

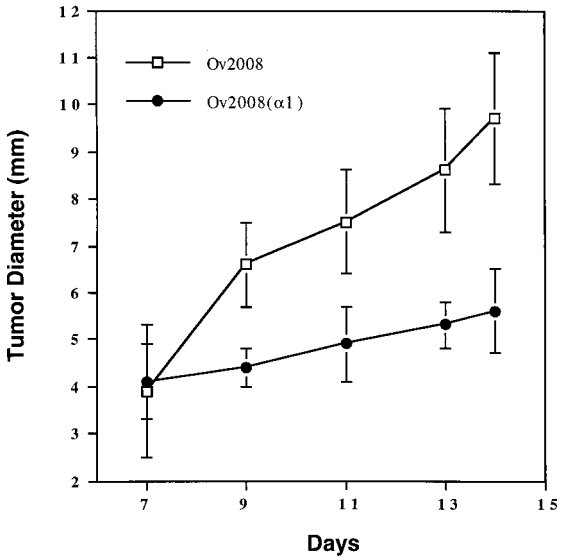

FIG. 9. Growth of Ov2008- and Ov2008(α1)-derived tumors following the administration of dexamethasone. Four nude mice were injected on opposite flanks with 5×10^6 Ov2008 and Ov2008(α1) cells. Tumors appeared in 4 to 5 days and grew to 3 to 4 mm in 7 days. From this point on, dexamethasone phosphate (10 μg) was injected daily to induce the expression of α-catenin in Ov2008(α1) cells. Tumor diameters were determined, and the collective data are presented as the means of results for four animals.

plexed with each other and localized to the cell membrane. Upon induction of wild-type α-catenin in Ov2008(α1) cells, the morphology was restored to that of typical epithelial cells, and α-catenin was found to both associate with the E-cadherin–β-catenin complex and localize to the membrane. These observations demonstrate that the other components of the cadherin-catenin complex in Ov2008 cells are functional and that the defect in cell adhesion in this cell line can be attributed to the deficiency in α-catenin. Deletion of the β-catenin binding domain by truncation of α-catenin, as seen in Ov2008 cells, may represent a novel mechanism whereby the E-cadherin cell adhesion system can become inactivated. Interestingly, Oyama et al. have described a signet ring cell carcinoma cell line in which cell adhesion has been perturbed as a result of an in-frame deletion in the β-catenin gene which disrupts the α-catenin binding domain (23). The consequence of the mutations in both of these cell lines is to break the essential link between E-cadherin and the actin cytoskeleton. Ov2008 cells express relatively low levels of the mutant form of α-catenin, and it has not yet been determined whether expression of higher levels of this mutant will result in an altered phenotype. It is an intriguing possibility that the mutant α-catenin expressed in Ov2008 cells, which retains 90% of the amino acid sequence, has additional functions independent of an association with β-catenin. The possibility that α-catenin mutations similar to that seen in Ov2008 cells are selected for in a subset of human tumors is currently under investigation.

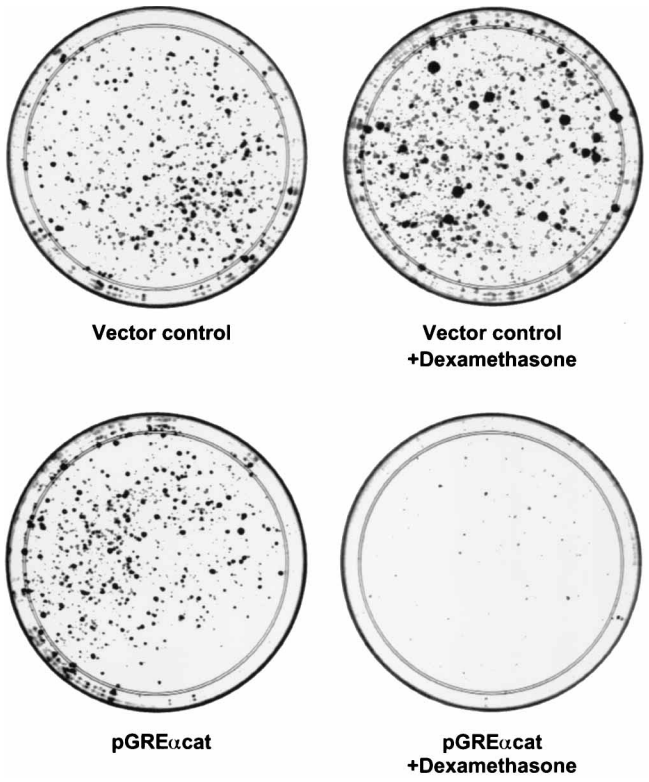

FIG. 8. Growth suppression assay of Ov2008 cells transfected with α-catenin. Ov2008 cells were transfected with 5 μg of either plasmid pGREαcat or the pGRE5-1/EBV vector alone. Forty-eight hours after transfection, 10^5 cells were plated in duplicate, 500 nM dexamethasone was added to half of the plates, and the colonies were selected for resistance to hygromycin B. Resistant colonies were stained 4 weeks after transfection, and representative plates are shown.

α-Catenin has been shown to act as an invasion suppressor in vitro (39), and the reduction of α-catenin correlates well with the metastatic potential of a tumor (7, 15, 38). Therefore, it has been proposed that this adhesion system functions to suppress metastasis in vivo. The results presented here suggest that restoring α-catenin and activating the E-cadherin-mediated cell adhesion system also plays a role in the regulation of cellular growth and tumorigenicity. Two previous reports have also suggested that the restoration of α-catenin expression in deficient cell lines negatively regulates cellular growth (2, 40). In the first of these reports, Watabe et al. documented a decrease in the growth rate of a single clone from an α-catenin-null lung carcinoma cell line in which constitutive α-catenin expression had been restored by

transfection (40). Subsequently, Ewing et al. introduced chromosome 5, which contains the α-catenin gene, into an α-catenin-null prostate cancer cell line by microcell-mediated transfer (2). Although they reported both growth and tumor suppression, which were correlated with the expression of α-catenin, the large number of other genes on chromosome 5, including the APC tumor suppressor gene, make a clear interpretation of this result difficult. In contrast to these previous studies, the inducible system for α-catenin expression described here has allowed the direct correlation of α-catenin expression with growth suppression both in vitro and in vivo. When α-catenin expression was induced, both the growth rate of Ov2008 cells in culture and their ability to form colonies were significantly repressed. Furthermore, a decrease in the expansion of actively growing tumors in vivo was shown to be coincident with the induction of α-catenin. Although the mechanism by which α-catenin and the cadherin-catenin cell adhesion system regulate cell growth remains unknown, the availability of an inducible system for α-catenin expression should facilitate the investigation of downstream effectors of this pathway.

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REFERENCES

1. **Aberle, H., S. Butz, J. Stappert, H. Weissig, R. Kemler, and H. Hoschuetzky.** 1994. Assembly of the cadherin-catenin complex *in vitro* with recombinant proteins. J. Cell Sci. **107:**3655–3663.
2. **Ewing, C. M., N. Ru, R. A. Morton, J. C. Robinson, M. J. Wheelock, K. R. Johnson, J. C. Barrett, and W. B. Isaacs.** 1995. Chromosome 5 suppresses tumorigenicity of PC3 prostate cancer cells: correlation with re-expression of α-catenin and restoration of E-cadherin function. Cancer Res. **55:**4813–4817.
3. **Herrenknecht, K., M. Ozawa, S. C. Ecker, F. Lottspeich, M. Lenter, and R. Kemler.** 1991. The uvomorulin-anchorage protein alpha catenin is a vinculin homologue. Proc. Natl. Acad. Sci. USA **88:**9156–9160.
4. **Hinck, L., I. S. Nathke, J. Papkoff, and W. J. Nelson.** 1994. Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. J. Cell Biol. **125:**1327–1340.
5. **Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White.** 1990. PCR protocols—a guide to methods and applications. Academic Press, Inc., San Diego, Calif.
6. **Jou, T.-S., D. B. Stewart, J. Stappert, W. J. Nelson, and J. A. Marrs.** 1995. Genetic and biochemical dissection of protein linkages in the cadherin-catenin complex. Proc. Natl. Acad. Sci. USA **92:**5067–5071.
7. **Kadowaki, T., H. Shiozaki, M. Inoue, S. Tamura, H. Oka, Y. Doki, K. Iihara, S. Matsui, T. Iwazawa, A. Nagafuchi, S. Tsukita, and T. Mori.** 1994. E-cadherin and α-catenin expression in human esophageal cancer. Cancer Res. **54:**291–296.
8. **Kintner, C.** 1992. Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain. Cell **69:**225–236.
9. **Kozak, M.** 1984. Selection of initiation sites by eukaryotic ribosomes: effect of inserting AUG triplets upstream from the coding sequence for preproinsulin. Nucleic Acids Res. **12:**3873–3893.
10. **Kozak, M.** 1992. Regulation of translation in eukaryotic systems. Annu. Rev. Cell Biol. **8:**197–225.
11. **Kozyraki, R., J.-Y. Scoazec, J.-F. Flejou, A. D'errico, P. Bedossa, B. Terris, M. Fiorentino, A.-F. Bringuier, W. Grigioni, and G. Feldmann.** 1996. Expression of cadherins and α-catenin in primary epithelial tumors of the liver. Gastroenterology **110:**1137–1149.
12. **Liu, C.-C., C. Simnsen, and A. Levinson.** 1984. Initiation of translation at internal AUG codons in mammalian cells. Nature **309:**82–85.
13. **Mader, S., and J. H. White.** 1993. A steroid-inducible promoter for the controlled overexpression of cloned genes in eukaryotic cells. Proc. Natl. Acad. Sci. USA **90:**5603–5607.
14. **Magee, A. I., and R. S. Buxton.** 1991. Transmembrane molecular assemblies regulated by the greater cadherin family. Curr. Opin. Cell Biol. **3:**854–861.
15. **Matsui, S., H. Shiozaki, M. Inoue, S. Tamura, Y. Doki, T. Kadowaki, T. Iwazawa, K. Shimaya, A. Nagafuchi, S. Tsukita, and T. Mori.** 1994. Immunohistochemical evaluation of alpha-catenin expression in human gastric cancer. Virchows Arch. **424:**375–381.
16. **Moll, R., M. Mitze, U. H. Frixen, and W. Birchmeir.** 1993. Differential loss of E-cadherin expression in infiltrating ductal and lobal breast carcinomas. Am. J. Pathol. **143:**1731–1742.
17. **Morton, R. A., C. M. Ewing, A. Nagafuchi, S. Tsukita, and W. B. Isaacs.** 1993. Reduction of E-cadherin levels and deletion of the α-catenin gene in human prostate cancer cells. Cancer Res. **53:**3585–3590.
18. **Nagafuchi, A., and M. Takeichi.** 1988. Cell binding function of E-cadherin is regulated by the cytoplasmic domain. EMBO J. **7:**3679–3684.
19. **Nagafuchi, A., and M. Takeichi.** 1989. Transmembrane control of cadherin-mediated cell adhesion: a 94-kD protein functionally associates with a specific region of the cytoplasmic domain of E-cadherin. Cell Regul. **1:**37–44.
20. **Nagafuchi, A., M. Takeichi, and S. Tsukita.** 1991. The 102-kD cadherin-associated protein: similarity to vinculin and posttranslational regulation of expression. Cell **65:**849–857.
21. **Ochiai, A., S. Akimoto, Y. Shimoyama, A. Nagafuchi, S. Tsukita, and S. Hirohashi.** 1994. Frequent loss of α-catenin expression in scirrhous carcinomas with scattered cell growth. Jpn. J. Cancer Res. **85:**266–273.
22. **Oda, T., Y. Kanai, Y. Shimoyama, A. Nagafuchi, S. Tsukita, and S. Hirohashi.** 1993. Cloning of the human α-catenin cDNA and its aberrant mRNA in a human cancer cell line. Biochem. Biophys. Res. Commun. **193:**897–904.
23. **Oyama, T., Y. Kanai, A. Ochiai, S. Akimoto, T. Oda, K. Yanagihara, A. Nagafuchi, S. Tsukita, S. Shibamoto, F. Ito, M. Takeichi, H. Matsuda, and S. Hirohashi.** 1994. A truncated β-catenin disrupts the interaction between E-cadherin and α-catenin: a cause of loss of intercellular adhesiveness in human cancer cell lines. Cancer Res. **54:**6282–6287.
24. **Ozawa, M., H. Baribault, and R. Kemler.** 1989. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. EMBO J. **8:**1711–1717.
25. **Ozawa, M., M. Ringwals, and R. Kemler.** 1990. Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. Proc. Natl. Acad. Sci. USA **87:**4246–4250.
26. **Peabody, D., S. Subramani, and P. Berg.** 1986. Effect of upstream reading frames on translation efficiency in simian virus 40 recombinants. Mol. Cell. Biol. **6:**2704–2711.
27. **Pierceall, W. E., A. S. Woodard, J. S. Morrow, D. Rimm, and E. R. Fearon.** 1995. Frequent alteration in E-cadherin and α-catenin expression in human breast cancer cell lines. Oncogene **11:**1319–1325.
28. **Reynolds, A. B., J. Daniel, P. D. McCrea, M. J. Wheelock, J. Wu, and Z. Zhang.** 1994. Identification of a new catenin: the tyrosine kinase substrate $p120^{cas}$ associates with E-cadherin complexes. Mol. Cell. Biol. **14:**8333–8342.
29. **Rimm, D. L., J. H. Sinard, and J. S. Morrow.** 1995. Reduced α-catenin and E-cadherin expression in breast cancer. Lab. Invest. **72:**506–512.
30. **Schipper, J. H., U. H. Frixen, J. Behrens, A. Unger, K. Jahnke, and W. Birchmeier.** 1991. E-cadherin expression in squamous cell carcinomas of head and neck: inverse correlation with tumor dedifferentiation and lymph node metastasis. Cancer Res. **51:**6328–6337.
31. **Shimazui, T., J. Schalken, L. Giroldi, C. Jansen, H. Akaza, K. Koiso, F. Debruyne, and P. Bringuier.** 1996. Prognostic value of cadherin-associated molecules (α-, β-, and γ-catenins and $p120^{cas}$) in bladder tumors. Cancer Res. **56:**4154–4158.
32. **Shiozaki, H., K. Iihara, H. Oka, T. Kadowaki, S. Matsui, J. Gofuku, M. Inoue, A. Nagafuchi, S. Tsukita, and T. Mori.** 1994. Immunohistochemical detection of α-catenin expression in human cancers. Am. J. Pathol. **144:**667–674.
33. **Takayama, T., H. Shiozaki, M. Inoue, S. Tamura, H. Oka, and T. Kadowaki.** 1994. Expression of E-cadherin and α-catenin molecules in human breast cancer tissues and association with clinicopathological features. Int. J. Oncol. **5:**775–780.
34. **Takayama, T., H. Shiozaki, S. Shibamoto, H. Oka, Y. Kimura, S. Tamura, M. Inoue, T. Monden, F. Ito, and M. Monden.** 1996. β-Catenin expression in human cancers. Am. J. Pathol. **148:**39–46.
35. **Takeichi, M.** 1990. Cadherins: a molecular family important in selective cell-cell adhesion. Annu. Rev. Biochem. **59:**237–252.
36. **Tanaka, M., and W. Herr.** 1990. Differential transcriptional activation of oct-1 and oct-2: independent activation domains induce oct-2 phosphorylation. Cell **60:**375–386.
37. **Tsukita, S., A. Nagafuchi, and S. Yonemura.** 1992. Molecular linkage between cadherins and actin filaments in cell-cell adherens junctions. Curr. Opin. Cell Biol. **4:**834–839.
38. **Vermeulen, S. J., E. A. Bruyneel, M. E. Bracke, G. K. De Bruyne, K. M. Vennekens, K. L. Vleminckx, G. J. Berx, F. M. van Roy, and M. M. Mareel.** 1995. Transition from the noninvasive to the invasive phenotype and loss of α-catenin in human colon cancer cells. Cancer Res. **55:**4722–4728.
39. **Vleminckx, K., L. Vakaet, M. Marell, W. Fiers, and F. Van Roy.** 1991. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell **66:**107–119.
40. **Watabe, M., A. Nagafuchi, S. Tsukita, and M. Takeichi.** 1994. Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin-catenin adhesion system in a dispersed carcinoma line. J. Cell Biol. **127:**247–256.