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Diverse Mechanisms of β-Catenin Deregulation in Ovarian Endometrioid Adenocarcinomas

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ABSTRACT

Clinical and molecular findings suggest that the four major histological subtypes of ovarian carcinoma (serous, clear cell, mucinous, and endometrioid) likely represent distinct disease entities. Prior studies have shown that ovarian endometrioid adenocarcinomas (OEAs) often carry mutations in the *CTNNB1* gene, which encodes β-catenin, a critical component of the Wnt signaling pathway. However, the nature of other defects in the Wnt signaling pathway in ovarian carcinomas remains largely unknown. Thus, in 45 primary OEAs and two OEA-derived cell lines, we sought to comprehensively address the prevalence of and mechanisms underlying β-catenin and Wnt pathway deregulation. *CTNNB1* missense mutations were detected in 14 primary tumors. All mutations affected the NH_2-terminal regulatory domain of β-catenin, presumably rendering the mutant proteins resistant to degradation. Immunohistochemical studies revealed nuclear accumulation of β-catenin in all but two tumors with *CTNNB1* mutations. Two primary tumors lacking *CTNNBI* mutations showed strong nuclear immunoreactivity for β-catenin. In one of the two tumors, biallelic inactivation of the *APC* gene was found. In the remaining 29 primary OEAs, unequivocal nuclear β-catenin immunoreactivity was not observed, though a nonsense mutation in *AXIN1* was observed in one tumor and a truncating frameshift mutation in *AXIN2* was seen in another case. Both OEA-derived cell lines studied (TOV-112D and MDAH-2774) had elevated constitutive T-cell factor/lymphoid enhancer factor transcriptional activity. TOV-112D cells were shown to harbor mutant β-catenin, whereas a missense *AXIN1* sequence alteration was identified in MDAH-2774 cells. Collectively, our findings demonstrate frequent defects of the Wnt signaling pathway in a particular subtype of ovarian carcinomas, *i.e.*, OEAs. Although mutations in the *CTNNB1* gene are the most common mechanism of β-catenin deregulation in OEAs, β-catenin deregulation may also result from mutations in the *APC*, *AXIN1*, and *AXIN2* genes.

INTRODUCTION

Ovarian cancer causes more deaths of women in the United States than any other gynecological malignancy (1). Most malignant ovarian tumors are epithelial (carcinomas) and are thought to arise from the ovarian surface epithelium or the secondary Müllerian system, which includes endometriosis (2). Although the existing classification scheme for ovarian carcinomas is based entirely on morphological criteria, clinical and molecular genetic analyses suggest that the major histological subtypes (*e.g.*, serous, clear cell, mucinous, and endometrioid) likely represent distinct disease entities (3–5). Moreover, ovarian carcinoma precursor lesions may well be subtype specific (6). Hence, a clearer understanding of ovarian cancer pathogenesis might be more readily attained from focused molecular genetic studies of important cell signaling pathways in distinct ovarian carcinoma subsets, rather than through studies of a more heterogeneous collection of carcinomas.

OEAs[3] share molecular genetic features with uterine endometrioid adenocarcinomas, including frequent mutations of the *PTEN* tumor suppressor gene (7), microsatellite instability (8, 9), and mutations of the *CTNNB1* gene (10–14). *CTNNB1* encodes β-catenin, a vertebrate homologue of *Drosophila* armadillo and a critical component of the highly conserved Wnt signaling pathway (15). The Wnt pathway has a critical role in regulating cell fate specification, proliferation, and differentiation in various tissues (15). β-Catenin levels are regulated by a protein complex containing the APC protein, GSK3β, and AXIN (16, 17). This protein complex promotes degradation of free cytosolic β-catenin via GSK3β-mediated phosphorylation of NH_2-terminal β-catenin sequences and subsequent ubiquitination and proteasome degradation of β-catenin. When the Wnt pathway is activated, GSK3β activity and β-catenin ubiquitination and degradation are inhibited, and β-catenin enters the nucleus where it complexes with TCF/LEF transcription regulator proteins (17). The TCF/LEF proteins cooperate with nuclear β-catenin to activate transcription of target genes, including perhaps c-*MYC* (18), *cyclin D1* (19, 20), and *MMP-7* (21), although many of the TCF/LEF target genes remain unknown. Oncogenic mechanisms leading to β-catenin stabilization include inactivation of the APC tumor suppressor protein and mutational activation of β-catenin itself via localized mutations of its NH_2-terminal GSK3β regulatory motif (22–24). More recently, inactivating mutations in *AXIN* (*AXIN1*) in hepatocellular carcinomas and colon cancer cell lines (25, 26), and inactivating mutations in its homologue, *AXIN2* (*AXIL/Conductin*), have been detected in microsatellite unstable colorectal cancers (27). Rare mutations of *CTNNG1*, which encodes γ-catenin, have also been identified in human cancer (28). γ-Catenin is another vertebrate homologue of *Drosophila* armadillo that, similar to β-catenin, functions in cell adhesion and Wnt signaling (29, 30). Although some prior studies have provided evidence that a sizable percentage of OEAs harbor β-catenin mutations (10–14), studies of *APC* in OEAs have been extremely limited thus far, and no *APC* mutations have been reported (31). Whether *CTNNG1*, *AXIN1*, or *AXIN2* mutations play a role in the pathogenesis of these tumors remains unknown.

In an effort to better understand the molecular pathogenesis of OEAs, we collected 45 primary OEAs and 2 OEA-derived cell lines and carried out a comprehensive molecular analysis of several genes encoding proteins known to function in the Wnt signaling pathway. We found Wnt pathway defects in both OEA cell lines and in nearly half of the primary OEAs analyzed. β-Catenin deregulation was most often attributable to mutation of β-catenin itself and less frequently to inactivating mutations in *APC*, *AXIN1*, or *AXIN2*. Collectively, our findings provide evidence for diverse mechanisms of β-catenin deregulation in this particular subtype of ovarian cancer.

MATERIALS AND METHODS

Tumor Samples. A total of 45 snap-frozen primary OEAs were analyzed. Six specimens were obtained from the Johns Hopkins Hospital; 2 from the

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[3] The abbreviations used are: OEA, ovarian endometrioid adenocarcinoma; APC, adenomatous polyposis coli; TCF/ LEF, T-cell factor/lymphoid enhancer factor; FBS, fetal bovine serum; PTT, protein truncation test.

University of Michigan Hospital; and 37 from the Cooperative Human Tissue Network/Gynecological Oncology Group Tissue Bank. A small portion of formalin-fixed, paraffin-embedded tissue from each specimen was histologically verified as OEA by a gynecological pathologist (K. R. C.) and classified as well, moderately, or poorly differentiated, based on the tumor's histological features. Tumor stage (I–IV) was assigned according to the International Federation of Gynecology and Obstetrics (FIGO) system. Analysis of tissues from human subjects was approved by the University of Michigan's Institutional Review Board (IRB-MED 2001-0022).

Cell Lines. Two OEA-derived cell lines (TOV-112D and MDAH-2774), two ovarian clear cell carcinoma-derived cell lines (TOV-21G and ES-2), two ovarian adenocarcinoma (histological type unknown)-derived cell lines (NIH: OVCAR-3 and SKOV-3), and colon cancer cell line SW480 were obtained from the American Type Culture Collection (Manassas, VA). Ovarian serous carcinoma-derived cell lines HOC-1, HOC-7, HOC-8, and ovarian serous cystadenoma cell lines ML3 and ML10 were a gift from L. Dubeau (USC School of Medicine, Los Angeles, CA). Ovarian carcinoma cell lines (histological type unknown) OVCAR-4, OVCAR-5, OVCAR-8, PEO1, and PEO4 were a gift of T. Hamilton (Fox Chase Cancer Center, Philadelphia, PA). Ovarian serous carcinoma cell line DOV13 was a gift of D. Fishman (Northwestern University, Chicago, IL). IOSE-80 cells (human ovarian surface epithelial cells expressing SV40 large T Antigen) were a gift of N. Auersperg (University of British Columbia, Vancouver, British Columbia, Canada). TOV-112D and TOV-21G were cultured in 1:1 mixture of MCDB 105 medium (Sigma Chemical Co., St. Louis, MO) and Medium 199 (Life Technologies, Inc., Gaithersburg, MD) with 15% FBS (Life Technologies, Inc.). MDAH-2774 cells were cultured in RPMI 1640/10% FBS. ES-2 cells were cultured in McCoy's 5A/10% FBS. All other cell lines were maintained in DMEM with 10% FBS.

DNA, RNA, and cDNA Preparation. Primary tumor tissues were manually microdissected prior to nucleic acid extraction to ensure that each tumor sample contained at least 70% tumor cells. H&E-stained sections of frozen tumor tissues were used as dissection guides. Genomic DNA was isolated from pooled frozen tissue sections, using standard SDS/proteinase K digestion followed by phenol/chloroform extraction. Total RNA was extracted with Trizol (Life Technologies, Inc.) according to the manufacturer's protocol. First-strand cDNA was synthesized from DNaseI-treated mRNA samples using random hexamer primers (Pharmacia Biotech, Piscataway, NJ) and Superscript II (Life Technologies, Inc.).

PCR. The PCR primers used for each reaction are specified in Table 1.

Table 1 *Primer sequences and annealing temperatures for PCR*

Gene	Product	Annealing temperature (°C)
CTNNB1		
F-CGTGGACAATGGCTACTCAA (exon 2)	F/R codon 5–82 in exon 3	54
R-TGCATACTGTCCATCAATA (exon 4)		
S-GGAGTTGGACATGGCCATGGAA (Sequencing primer)		
CTNNG1		
F-GCCACGATGGAGGTGATGAA	F/R codon 1–58 in exon 1	62
R-CCTGGGTGTAAGTGGTGGTT		
APC		
T7-trans: GGATCCTAATACGACTCACTATAGGGAGACCACCATGG		
F-T7-trans-GTCCAAGGGTAGCCAAGG	F/R codon 1–796	58
R-CATCATGTCGATTGGTGTCA		
F1-T7-trans-ATGCATGTGGAACTTTGTGG	F1/R1 codon 686–1211	58
R1-GAGGATCCATTAGATGAAGGTGTGGACG		
F2-T7-trans-TTTCTCCATACAGGTCACGG	F2/R2 codon 1106–1681	56
R2-CTGTAGGAATGGTATCTCG		
F3-T7-trans-CAGGAAAATGACAATGGGAATGA	F3/R3 codon 1537–2247	56
R3-CTAGGGCTTTTGGAGGCTGGAGT		
AXIN1		
F1-CTAATCCTTGTCCTCTTAATGG	F1/R1 exon 1	60
R1-CTGAACTCTCTGCCTTCGCTGTAC		
F2-TAACATGCCCTGCTTGCCTG	F2/R2 exon 2	62
R2-GTATCCGGGGCGGGACTT		
F3-TAACGGCTGCCTCTTTCTCC	F3R3 exon 3	64
R3-CTGAGGACCGCAAAGCCGGT		
F4-GTGCCTCACAGTCCTGTCC	F4/R4 exon 4	62
R4-GCAAGAAAACAGCACGACACC		
F5-TGGTGCTGCTTGTCCCCAC	F5/R5 exon 5	64
R5-TCCTCACTGACAGGCGCAC		
F6-CTCGCAGCTCTTGCCCTTG	F6/R6 exon 6	62
R6-CTGGCTGCGTGCGGGGTG		
F7-TCACAACTTGTTCTCTTGTTCC	F7/R7 exon 7	60
R7-ACCCCACGACGCGGCCGT		
F8-CCTTTGACGCGGGTTGTTCC	F8/R8 exon 8	62
R8-GGGAGGACCCTCAGGACG		
F9-CCACCGCTGCATCTCTTTCC	F9/R9 exon 9	66
R9-GGGGCACCCCAGCCCTC		
F10-CCTCCTCACCTCCGTCCTG	F10/R10 exon 10	66
R10-CGGCCAGCCCACCAGCCT		
AXIN2		
S1-GCCGATTGCTGAGAGGAACT	S1/AS1 codon 1–165	62
AS1-CGGTCTGCGCCTGGTCAAA		
S2-GCCACCAAGACCTACATAAGA	S2/AS2 codon 159–292	62
AS2-TCACTGGATATCTCACTGTCG		
S3-GGTTCTGGCTATGTCTTTGC	S3/AS3 codon 292–427	60
AS3-GTATCGTCTGCGGGTCTTC		
S4-CACCCCCTCTCCCTACTGC	S4/AS4 codon 427–564	60
AS4-GCCTCTGCTGCCGCCAAAC		
S5-GGGGGCAGCGAGTATTACT	S5/AS5 codon 551–683	60
AS5-GCGTGTTGGGTGGGGTCA		
F-CAAAGCACAAAAAAGGCCTACC	F/R codon 645–712	60
R-GATTCCTGTCCCTCTGCTGAC		
S6-CCCTGACCCCACCCAACA	S6/AS6 codon 693–843	60
AS6-CCAACAGTTCACCAAAGCCA		

GenBank accession numbers for the nucleotide sequences used to determine suitable primer sequences are as follows: *CTNNB1*, X87838; *CTNNG1*, AF233882; *APC*, M74088; *AXIN1*, AF009674; and *AXIN2*, AF078165. The entirety of *CTNNB1* exon 3 was amplified from cDNA using a forward primer in exon 2 and reverse primer in exon 4. A portion of *CTNNG1* (γ-catenin) exon 1 was amplified from genomic DNA using exon-based primers flanking the region encoding the GSK3β regulatory domain near the γ-catenin NH_2 terminus. The coding region of *AXIN1* was amplified from genomic DNA exon-by-exon using 10 pairs of intron-based primers. The coding region of *AXIN2* was amplified from cDNA using primers to generate six overlapping cDNA segments. PCR for *APC* was performed using either cDNA (exons 1–14) or genomic DNA (exon 15) as templates. All PCR reactions were carried out in a final volume of 50 μl containing 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 200 μM of each deoxynucleotide triphosphate, 0.2 μM each primer, and 2.5 units Taq polymerase (Life Technologies, Inc.). The annealing temperature used for each primer pair is specified in Table 1. After an initial denaturation at 95°C for 3 min, templates were amplified for 35 cycles (95°C for 30 s, 54–66°C for 30 s, and 72°C for 45 to 90 s), followed by a 5-min extension at 72°C. PCR products were visualized on 1- 2% agarose gels and purified with Qiaquick gel extraction kits (Qiagen, Valencia, CA).

Nucleotide Sequence Analysis. Sequencing of PCR products was carried out using Thermo-Sequenase and ^{33}P-labeled dideoxynucleoside triphosphates (United States Biochemical, Cleveland, OH) or by automated sequencing conducted by the University of Michigan's Sequencing Core. All sequence alterations were confirmed by bidirectional manual sequencing of PCR products generated by at least two independent reactions.

PCR Amplification of Microsatellite Sequences. Two microsatellite loci on chromosome 11q23 (*D11S1647* and *D11S1987*; Research Genetics, Huntsville, AL) were amplified from genomic DNA extracted from tumor and matched normal tissue. Each PCR mixture contained 37.5 mM Tris, 2.2 mM $MgCl_2$, 200 μM each dATP, dGTP, dTTP, 25 μM dCTP, 2 μCi (3000 Ci/mmol) [^{32}P]dCTP, 1 μM each primer, and 1 unit of Taq polymerase. Target sequences were amplified for 30 cycles as described above. PCR products were resolved by electrophoresis on 6% polyacrylamide gels, which were dried and subjected to autoradiography.

PTT. A PTT was used to screen samples for protein truncating mutations in the *APC* gene. A large portion of the *APC* coding region (codons 1–2247; exons 1–14 and a portion of exon 15) was amplified from cDNA or genomic DNA (exon 15) in four overlapping segments encompassed by the T7-modified primers (T7-trans) specified in Table 1. The great majority of the *APC* mutations identified to date are within this region of the gene.[4] After verifying the presence of PCR-amplified products for each segment on 1% agarose gels, radioactive *in vitro* transcription/translation of the PCR-generated fragments was performed using the TNT Quick Coupled Transcription/Translation System (Promega Corp., Madison, WI) according to the manufacturer's instructions. Products were separated on NuPAGE 10% Bis-Tris precast gels (Invitrogen, San Diego, CA) at 40 V for 60 min. The gels were fixed in methanol and acetic acid for 30 min and then soaked in Amplifying Solution (Amersham, Arlington Heights, IL) for 30 min for fluorographic enhancement of signal. After fixation in 7% acetic acid/7% methanol/1% glycerol, gels were dried and exposed to X-OMAT AR scientific imaging film (Kodak, Rochester NY). For samples with bandshifts in the PTT, the appropriate *APC* gene regions were amplified by PCR and bidirectionally sequenced.

Immunohistochemical Analysis of β-Catenin. Five-μm sections of formalin-fixed, paraffin-embedded tissues were mounted on Probe-On slides (Fisher Scientific, Itasca, IL), deparaffinized in xylene, and then rehydrated into distilled H_2O through graded alcohols. Antigen retrieval was enhanced by microwaving the slides in citrate buffer (pH 6.0; Biogenex, San Ramon, CA) for 10 min. Endogenous peroxidase activity was quenched by incubation with 6% hydrogen peroxide in methanol, and then the sections were postfixed in 10% buffered formalin, washed, and blocked with 1.5% normal horse serum for 1 h. Sections were then incubated with a mouse monoclonal anti-β-catenin antibody (C19220; Transduction Laboratories, Lexington, KY) at a dilution of 1:500 overnight at 4°C. Slides were washed in PBS and then incubated with a biotinylated horse antimouse secondary antibody for 30 min at room temperature. Antigen-antibody complexes were detected with the avidin-biotin peroxidase method using 3,3′-diaminobenzidine as a chromogenic substrate (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Immunostained sections were lightly counterstained with hematoxylin and then examined by light microscopy. Immunostaining was scored on a three-tiered scale for intensity (+, absent/weak; ++, moderate; and +++, strong) in the tumor cell nuclei, cytoplasm, and cell membranes.

Immunofluorescence Studies of β-catenin. Cells were grown on coverslips for 1–2 days, then fixed with 3% paraformaldehyde for 15 min at room temperature, and permeabilized with 1% goat serum/0.5% Triton X-100/PBS for 15 min at room temperature. After washing with PBS, the slides were blocked with 20% goat serum/0.2% Triton X-100/PBS for 30 min at room temperature. Cells were incubated with anti-β-catenin antibody (C19220; Transduction Laboratories) diluted 1:300 at room temperature for 2 h. After washing with 2% goat serum/0.2% Triton-X-100/PBS, cells were incubated with fluorescein (FITC)-conjugated AffiniPure donkey antimouse IgG (Jackson Immuno Research Laboratories, Inc., West Grove, PA) at a dilution of 1:200 for 60 min at room temperature. Slides were washed with PBS and then counterstained with propidium iodide (Sigma Chemical Co., St. Louis, MO) at a concentration of 1 μg/ml for 5 min. After mounting in aqueous medium, fluorescent microscopy was used to observe β-catenin localization.

β-Catenin/TCF Transcription Reporter Assay. β-Catenin/TCF transcriptional reporter assays were performed essentially as described previously (24, 32). To measure TCF reporter activity in the ovarian carcinoma and other cell lines, cultured cells were plated in six-well plates 16 h prior to transient transfection with the reporter constructs pTopflash or pFopflash, which were gifts from B. Vogelstein (Johns Hopkins University, Baltimore, MD). All transfections were performed with FuGene 6 reagent (Roche, Indianapolis, IN) and 0.5 μg of pTopflash or pFopflash. To normalize transfection efficiency in the reporter assays, cells were cotransfected with 0.5 μg of pCH110 (Amersham), which contains a functional *LacZ* gene cloned downstream of a cytomegalovirus early region promoter-enhancer element. Forty-eight h after transfection, the cells were washed with PBS and then lysed with reporter lysis buffer (Promega). Luciferase activities were measured with a luminometer (model TD-20E; Turner Corp., Mountain View, CA) after adding luciferase assay reagent (Promega) to the cell lysates. β-galactosidase activities were measured using the β-galactosidase enzyme assay system (Promega) and a microplate reader (model 3550-UV; Bio-Rad, Hercules, CA). Each experiment was performed in triplicate.

RESULTS

TCF-dependent Transcription Is Activated in OEA-derived Cell Lines. Most available ovarian cancer cell lines were derived from serous carcinomas, not endometrioid, clear cell, or mucinous primary tumors. In some cases, the histological subtype of primary carcinoma from which a cell line was derived was unknown, but the origin was most likely serous, because serous carcinomas are more common than all other histological subtypes combined (33). We assayed TCF transcriptional activity as a strategy for assessing alterations in the Wnt signaling pathway in ovarian carcinoma cell lines, two of which are known to be derived from OEAs (TOV-112D and MDAH-2774). Cells were transfected with either a wild-type TCF-dependent luciferase reporter plasmid (pTopflash) or a mutated control version (pFopflash), and the ratio of the luciferase activity of pTopflash *versus* pFopflash in a given cell line was considered a measure of relative TCF activity. Representative data are shown in Fig. 1. Both TOV-112D and MDAH-2774 demonstrated constitutively increased TCF-dependent transcriptional activity (about 30- and 7-fold, respectively) compared with ovarian surface epithelial cells (IOSE-80), serous cystadenoma-derived cells (ML3 and ML10), or various cell lines derived from nonendometrioid ovarian carcinomas (TOV-21G, ES-2, DOV13, HOC-1, HOC-7, HOC-8, A1847, NIH: OVCAR-3, OVCAR4, OVCAR5, OVCAR8, OVCAR10, PEO1, PEO4, and SKOV3). Increased (5.3-fold) TCF-dependent transcription in MDAH-2774 cells has been noted previously by Furlong and Morin (34).

[4] Internet address: http://archive.uwcm.ac.uk/uwcm/mg/ns/1/119682.html.

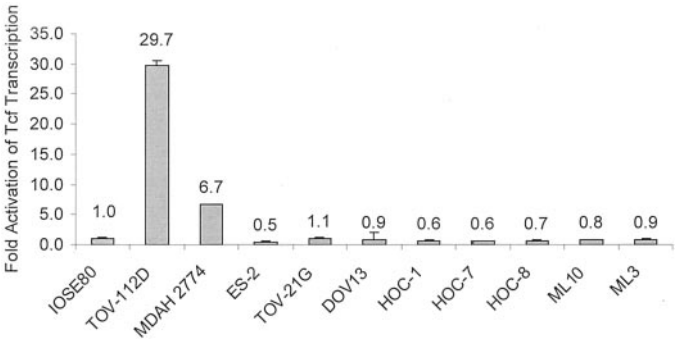

Fig. 1. Activation of TCF-dependent transcription in TOV-112D and MDAH-2774 endometrioid adenocarcinoma cells. The ratio of luciferase activities from a TCF-responsive reporter (pTopFlash) *versus* a control luciferase reporter gene construct (pFopFlash) was determined 48 h after transfection with FuGene6. Luciferase activities were normalized for transfection efficiency by cotransfection with a β-galactosidase-expressing vector. TCF-dependent transcription in two endometrioid adenocarcinoma-derived cell lines (TOV-112D and MDAH-2774), ovarian surface epithelial cells (IOSE-80), serous cystadenoma-derived cell lines (ML3 and ML10), clear cell carcinoma-derived cell lines (TOV-21G and ES-2), and serous carcinoma-derived cell lines (DOV13, HOC-1, HOC-7, and HOC-8) are shown. The data represent mean values from triplicate experiments; *bars*, SD.

Nuclear Localization of β-Catenin in OEA Lines and a Subset of Primary Tumors. Immunofluorescent staining of the two OEA-derived cell lines, TOV-112D and MDAH-2774, showed nuclear accumulation of β-catenin in a subset of tumor cells, similar to the pattern of β-catenin staining observed in cell lines with known Wnt pathway defects (Fig. 2, *A–D*). Nuclear immunoreactivity for β-catenin was more diffuse in TOV-112D than in MDAH-2774 cells. The basis for nonuniformity of nuclear β-catenin accumulation in cell lines with pathway defects is unclear, but differences may relate to nonuniformity of β-catenin expression in individual cells and/or to the levels of other proteins, such as ICAT, that may regulate β-catenin or its interaction with TCF/LEF factors (35). Nuclear immunoreactivity for β-catenin was also observed in 14 of the 45 primary OEAs (Table 2 and Fig. 2, *E–J*). Primary tumors with nuclear accumulation of β-catenin frequently showed reduced membrane staining. Similar to the situation in cell lines, nuclear localization was typically noted in many, but not all, neoplastic cells within a given tumor.

***CTNNB1* and *CTNNG1* Mutational Analyses.** Somatic missense mutations in *CTNNB1* sequences encoding the NH_2-terminal portion of β-catenin were identified in 14 of 45 (31%) primary OEAs. All tumors with mutations were well or moderately differentiated, and many showed squamous differentiation. *CTNNB1* mutations affected serine (or immediately adjacent) residues in the presumptive GSK3β regulatory motif at the β-catenin NH_2 terminus (Table 2 and Fig. 3; Ref. 16). A missense *CTNNB1* mutation at codon 37 (Ser→Ala) was also found in the OEA-derived cell line TOV-112D (Fig. 3). Twelve of the 14 primary OEAs with *CTNNB1* mutations showed nuclear accumulation of β-catenin protein (Table 2). Exon 1 of *CTNNG1*, which encodes γ-catenin sequences that are also presumptive targets for phosphorylation by GSK3β, was sequenced in all primary OEAs lacking *CTNNB1* mutations and in MDAH-2774 cells. No *CTNNG1* mutations were identified.

APC Mutational Analysis. PTTs for *APC* mutations were performed on 3 primary tumors; 2 with nuclear β-catenin accumulation and no demonstrable *CTNNB1* mutation (OE-13T and OE-32T), and one with moderate cytoplasmic but no detectable membrane staining for β-catenin (OE-15T). The OEA-derived MDAH-2774 cell line was also analyzed for truncating mutations in *APC*. Four overlapping gene segments spanning *APC* codons 1–2247 were evaluated. One tumor (OE-32T) showed aberrantly migrating translation products from the first (codons 1–796) and second (codons 686-1211) segments in

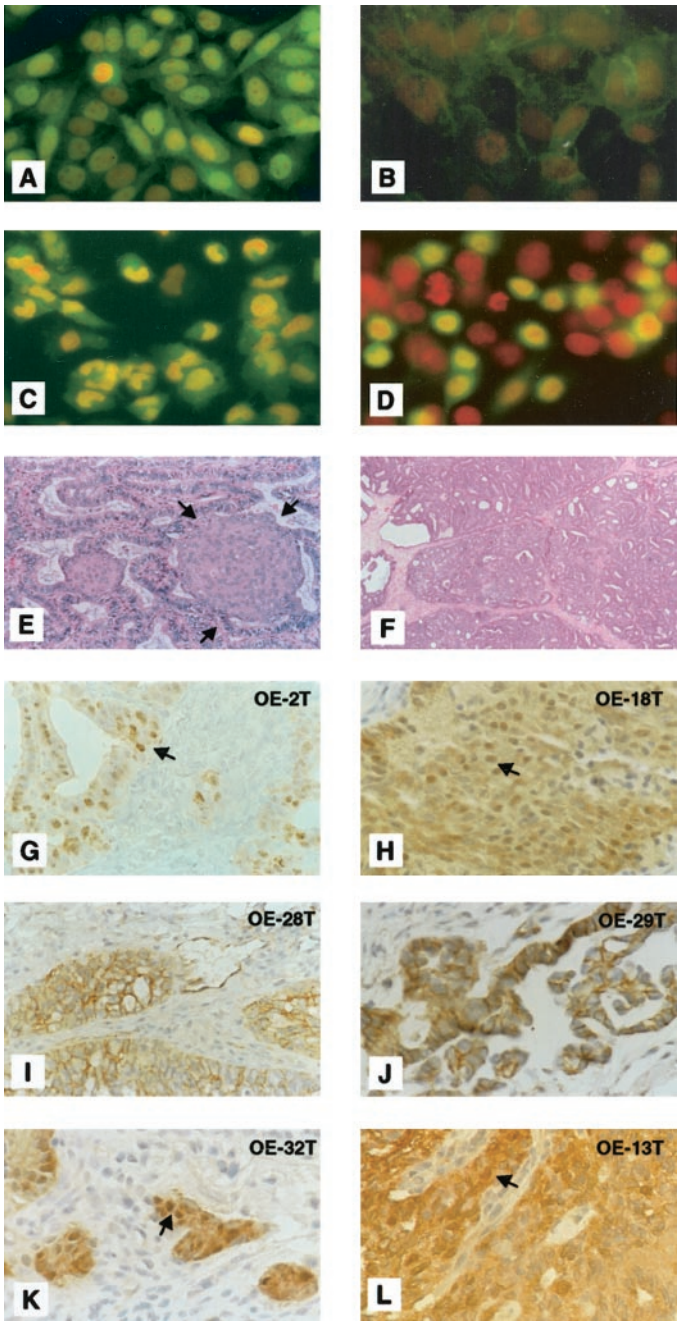

Fig. 2. Immunofluorescent and immunohistochemical staining of β-catenin in OEAs. Immunofluorescent staining of β-catenin in cell lines: *A*, nuclear accumulation of β-catenin in SW480 colorectal carcinoma cells with no wild-type APC and mutant APC allele; *B*, IOSE-80 cells (ovarian surface epithelial cells with SV40 large T antigen and intact β-catenin/TCF signaling pathway) showing membrane localization of β-catenin; *C*, nuclear accumulation of β-catenin in TOV-112D OEA cells with mutant β-catenin; and *D*, MDAH-2774 OEA cells with *AXIN1* sequence alteration. Representative H&E-stained sections of primary OEAs: *E*, well-differentiated OEA showing focal squamous differentiation (*arrows*); *F*, poorly differentiated OEA. Immunohistochemical staining of β-catenin in primary OEAs: *G*, representative OEA with β-catenin mutation (OE-2T, see Table 2) showing prominent nuclear accumulation of β-catenin in tumor cell nuclei (*arrow*), with little cytoplasmic or membrane-associated staining; *H*, representative OEA with β-catenin mutation (OE-18T) showing both nuclear (*arrow*) and cytoplasmic accumulation of β-catenin in region of tumor with squamous differentiation; *I* and *J*, representative OEAs without β-catenin mutation (OE-28T and OE-29T) showing membrane-associated localization of β-catenin immunoreactivity without detectable nuclear immunoreactivity; *K* and *L*, two OEAs (OE-32T and OE-13T) lacking demonstrable β-catenin mutations but showing prominent nuclear and/or cytoplasmic immunoreactivity for β-catenin in the vast majority of neoplastic cells.

Table 2 *Summary of β-catenin immunodetection and sequence analysis of Wnt pathway genes in OEAs*

Sample ID		Clinical data			β-catenin IH[b]			*CTNNB1* mutation (exon 3)			*APC* mutation (codons 1–2337)			*AXIN1* mutation			*AXIN2* mutation		
Primary	Tumors	Stage	Grade[a]	Age	N	C	M	Nucleotide	AA	Codon	Nucleotide	AA	Codon	Nucleotide	AA	Codon	Nucleotide	Codon	Type
1	1T	III	PD	60		+													
2	2T	I	WD	65	+++			GGA-CGA	Gly-Arg	34									
3	5T	IIIC	PD	58		+	+										2083, del G	G665X	frameshift
4	6T	III	PD	45		++	++												
5	7T	IIIC	PD	69		+	+												
6	8T	III	PD	73		++	++												
7	11T	IIB	PD	43		+													
8	12T	IC	WD	77			+												
9	13T	IA	MD	39	+	+													
10	14T	IV	MD	50		+	+++	TCT-TGT	Ser-Cys	33									
11	15T	IC	PD	61		++													
12	16T	I	WD	48			+												
13	17T	IC	WD	67	+	+		TCT-TGT	Ser-Cys	33									
14	18T	IIA	WD	35	++	++		TCT-TAT	Ser-Tyr	33									
15	19T	IA	WD	50			++	TCT-TAT	Ser-Tyr	45									
16	20T	IV	MD	69			++												
17	21T	IIIB	WD	52	+	+		GGA-GAA	Gly-Glu	34									
18	22T	IIB	WD	53	+	+		GAC-TAC	Asp-Tyr	32									
19	23T	IIIC	PD	68		++	++												
20	24T	IIIC	WD	66			++												
21	25T	IIIC	PD	58		+	+												
22	26T	IIIC	MD	48		+	+												
23	28T	IV	PD	73		+	+												
24	29T	IIIC	MD	72		+	++							AAG-TAG	Lys-Stop	416			
25	30T	IC	WD	51		+	+												
26	31T	IIIC	PD	49			+												
27	32T	IC	WD	47	++	+					CGA-TGA	Arg-Stop	554, 1114						
28	33T	IB	PD	N/A		+	+												
29	34T	IIA	PD	48		+													
30	35T	IV	PD	70		+	+												
31	36T	IIA	WD	45	++	++		GAC-CAC	Asp-His	32									
32	37T	N/A	WD	N/A	+	+		TCT-TGT	Ser-Cys	37									
33	38T	IIIC	MD	49		+	+												
34	39T	IIIC	MD	73		++	+++												
35	40T	IIIC	PD	58			+												
36	42T	IIIC	MD	61			+												
37	43T	IIIC	MD	43		+	+												
38	44T	I	WD	69	+	+	+++	TCT-TGT	Ser-Cys	33									
39	45T	N/A	MD	N/A	++	++	+	GAC-CAC	Asp-His	32									
40	46T	N/A	PD	N/A		+	+												
41	47T	IA	WD	72	++			TCT-GCT	Ser-Ala	37									
42	48T	IC	WD	N/A	+	+	+	GGA-GAA	Gly-Glu	34									
43	49T	IIIC	MD	53		+	++												
44	71T	IC	MD	49	++			GGA-GTA	Gly-Val	34									
45	72T	III	MD	52			+												
Cell lines																			
1	MDAH2774													GTA-ATA	Val-Ile	555			
2	TOV-112D							TCT-GCT	Ser-Ala	37									

[a] WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; N/A, not available.
[b] IH, immunohistochemistry. +, absent/weak; ++, moderate; +++, strong. N, cell nuclei; C, cytoplasm; M, cell membranes.

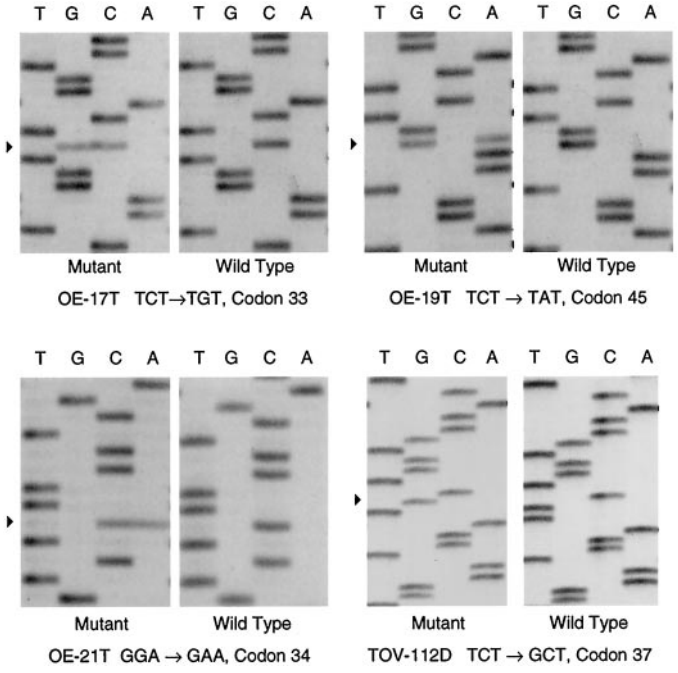

Fig. 3. β-Catenin mutations in representative primary OEAs and TOV-112D cells. Exon 3 mutations in 3 different tumors and TOV-112D cells are shown (*arrows*, mutant bands). For each tumor sample, the wild-type sequence is shown at *right*. For primary tumor samples virtually devoid of nonneoplastic cells, the bands from wild-type and mutant alleles should be of equal intensity. The reduced intensity of the mutant allele relative to wild type in some tumors likely reflects contamination of primary tumor DNA by nonneoplastic cells.

addition to the wild-type protein product (Fig. 4A and data not shown). Sequence analysis of OE-32T DNA revealed nonsense mutations at codons 554 and 1114; both mutations resulted in the conversion of an arginine residue to a stop codon (Fig. 4*B*).

***AXIN1* Mutational Analysis.** Interactions between AXIN and APC and AXIN and β-catenin are essential for promoting β-catenin phosphorylation and degradation (36). All primary OEAs with wild-type *CTNNB1* and *APC* genes were evaluated for mutations in exons 1–5 of *AXIN1*. Although studies to date are somewhat limited, all previously identified *AXIN1* mutations in human cancers have been shown to occur within these exons, which encode the domains implicated in binding APC, GSK3β, and β-catenin (Fig. 5*A*; Ref. 26). The entirety of the *AXIN1* coding region was examined in MDAH-2774 cells and in the 2 primary tumors with wild-type *CTNNB1* sequences and nuclear β-catenin accumulation (OE-13T and OE-32T) as well as the tumor with significantly increased cytoplasmic β-catenin staining but no membrane staining (OE-15T). A protein truncating mutation in *AXIN1* (exon 4, codon 416, Lys→stop) was identified in tumor OE-29T (Fig. 5*B*), and a missense sequence alteration in *AXIN1* (exon 5, codon 555, Val→Ile) was identified in MDAH-2774 cells (Fig. 5*C*). Although this is a conservative amino acid change, the mutation affects a residue predicted to lie within the region of *AXIN1* thought to bind β-catenin (26). Three silent single-nucleotide polymorphisms within *AXIN1* were also found, affecting nucleotide 93 (codon 31) in exon 1, nucleotide 873 (codon 291) in exon 1, and nucleotide 2517 (codon 839) in exon 9. The codon 31 and 291 single-nucleotide polymorphisms and several other polymorphisms in *AXIN1* have been reported previously (37).

***AXIN2* Mutational Analysis.** Previous studies of colorectal carcinomas have shown *AXIN2* mutations in four mononucleotide repeats within exon 7 (27). These mutations were found only in colorectal cancers with defective mismatch repair and were not seen in mismatch repair-proficient tumors. Hence, the region encompassing these mononucleotide tracts (codons 640–718) was evaluated in all primary OEAs with wild-type *CTNNB1* alleles. In the 3 primary OEAs with abnormal β-catenin levels and/or localization and no detectable *CTNNB1* mutations (OE-13T, OE-32T, and OE-15T), the entire *AXIN2* coding region was sequenced. A

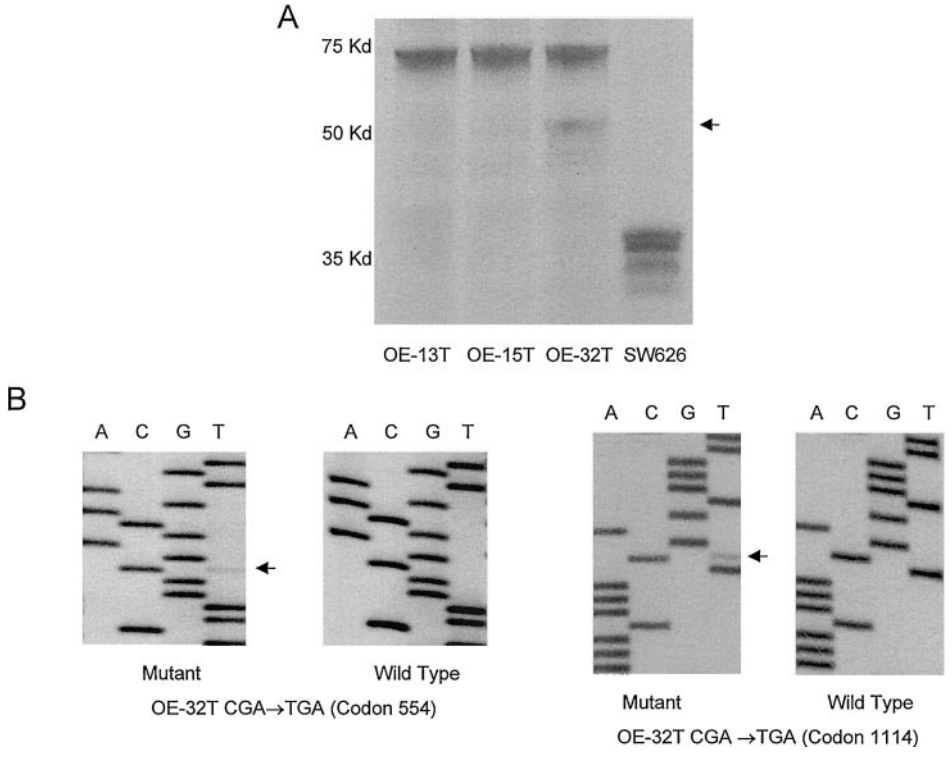

Fig. 4. APC gene mutations in OE-32T. *A*, representative PTT of *APC* (segment 2, codons 686-1211) showing a truncated protein product (*arrow*) in OE-32T. For this APC segment, the control cell line is SW626, which contains no wild-type APC and an insertion of one adenine at nucleotide 2941, predicted to produce a frameshift and downstream termination codon. *B*, sequence of the *APC* gene in OE-32T genomic DNA, showing mutations (*arrows*) at codons 554 and 1114, each predicting conversion of an Arg residue (CGA) to a stop codon (TGA). The two mutations presumably affect different *APC* alleles. The wild-type sequence from matched normal tissue is shown on the *right*.

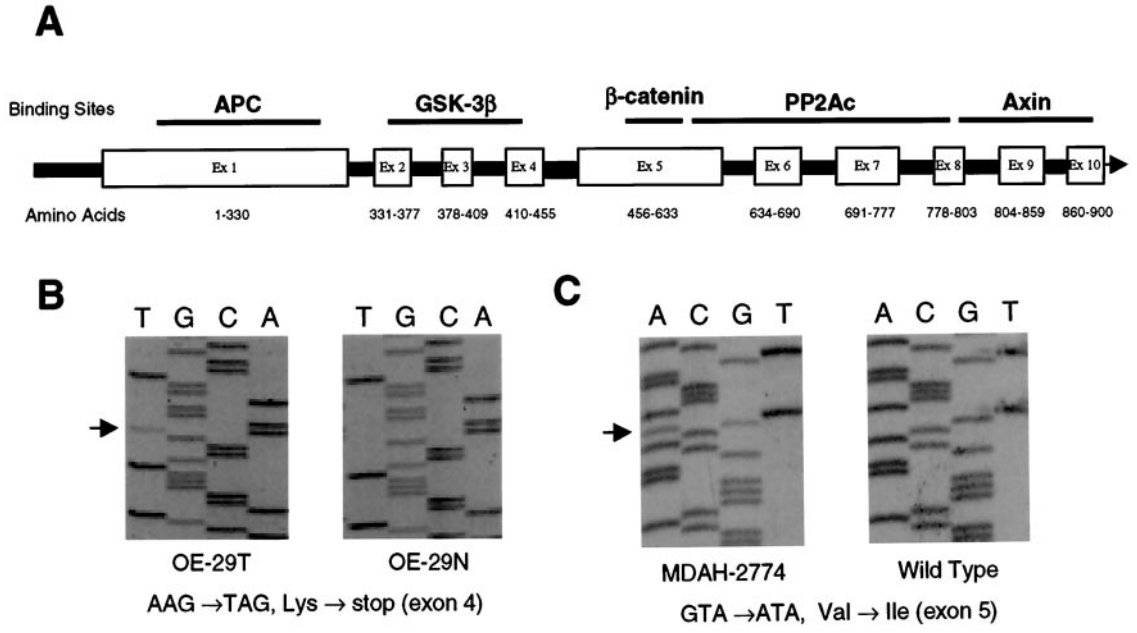

Fig. 5. AXIN1 structure and mutations. *A*, schematic diagram of *AXIN1* genomic structure and potential interaction domains. The sequence data shown are derived from partial human *AXIN1* mRNA and protein sequences (GenBank and Swissprot accession numbers AF009674 and 015169) and human genomic sequence (GenBank accession numbers Z69667, Z98272, and Z99754). The initiation codon for *AXIN1* has not been identified (17). Hence, the amino acid sequence shown is numbered according to the available database sequence with the amino acid residue specified by the first three nucleotides of the mRNA sequence/open reading frame arbitrarily designated 1*. *Bold lines above* the genomic structure indicate the coding regions corresponding to each protein binding site. *PP2Ac*, protein phosphatase 2Ac. Figure modified from Satoh *et al.* (26). *B*, nonsense mutation of *AXIN1* in primary tumor OE-29T. Portion of *AXIN1* exon 4 sequence in OE-29T (*left*) and matched normal tissue OE-29N (*right*). *Arrow*, mutation (AAG-TAG). The signal intensity of the wild-type *versus* mutant bands in the tumor DNA suggests the other *AXIN1* allele in the tumor DNA is wild type at the sequences shown, even assuming that some percentages of the wild-type sequences were contributed by contaminating nonneoplastic cells. *C*, missense mutation of *AXIN1* in OEA-derived cell line MDAH-2774. *Arrow*, mutation (GTA-ATA). The sequence shown is in the reverse orientation.

frameshift mutation at codon 665 (single G deletion) was found in one primary tumor (OE-5T; Fig. 6*A*). In previous work (38), this tumor was found to exhibit instability at two of two microsatellite sequences (Fig. 6*B*).

DISCUSSION

Mutations of NH_2-terminal β-catenin sequences have been demonstrated in various human tumor types, including colorectal carcinomas (24, 39), melanomas (40), hepatoblastomas (41), medulloblastomas (42), prostatic carcinomas (43, 44), and uterine endometrial carcinomas (45–48), particularly endometrioid adenocarcinomas with squamous differentiation (10). Prior studies of ovarian carcinomas have indicated that β-catenin mutations are common only in OEAs, with estimated mutation frequencies of 16–54% (10–14).

We have pursued our studies of the prevalence and mechanisms underlying β-catenin deregulation in 45 histologically verified frozen primary OEA tissues. This is, in fact, a substantial number of primary tumors of this particular histological subtype, because only 10–20% of ovarian carcinomas are endometrioid (49). We identified *CTNNB1* exon 3 mutations in 31% of our primary ovarian endometrioid carcinomas. Mutations inactivating the *APC*, *AXIN1*, and *AXIN2* genes were each observed in 1 primary OEA and were found in tumors that showed wild-type *CTNNB1* alleles. One of the 45 tumors we studied exhibited nuclear β-catenin accumulation not accounted for by mutations in the *CTNNB1*, *APC*, *AXIN1*, or *AXIN2* genes. To our knowledge, our study is the first to identify mutations of *APC*, *AXIN1*, or *AXIN2* in ovarian carcinomas. Collectively, our findings provide evidence for diverse mechanisms of β-catenin deregulation in OEAs, with demonstrable genetic mechanisms leading to β-catenin deregulation present in roughly 40% of our primary OEA specimens and in both OEA-derived cell lines. Interestingly, we observed that 11 of the 14 OEAs with mutant β-catenin were well differentiated, and none were poorly differentiated. Our findings are consistent with prior reports that OEAs with mutant β-catenin are more frequently low

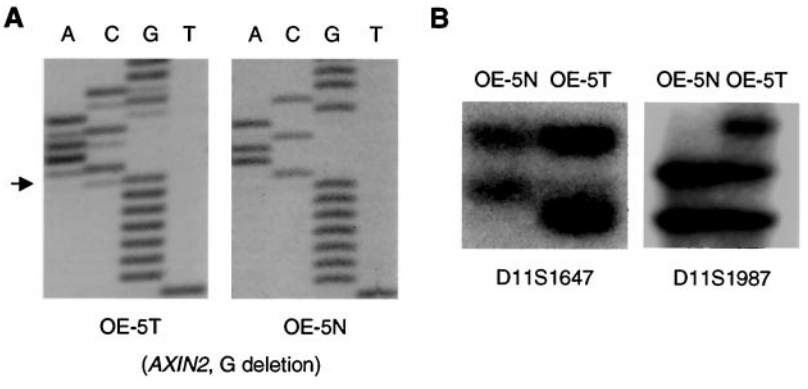

Fig. 6. AXIN2 mutation in primary OEA with microsatellite instability. *A*, *AXIN2* mutation in primary tumor OE-5T. Single nucleotide (G) deletion in short polyG tract is present in tumor DNA (*left*) but not in matched normal DNA (*right*). The mutation results in a frameshift, as indicated by the sequencing pattern above the site of the mutation. *B*, instability of microsatellite sequences in primary tumor OE-5T. The tumor DNA shows an upward shift at *D11S1647* and downward shift at *D11S1987* compared with matched normal DNA.

grade, exhibit squamous differentiation, and are associated with a favorable prognosis (10, 12, 13).

Furlong *et al.* (50) identified a frameshift mutation of *APC* exon 15 in SW626, a cell line originally thought to be of ovarian origin. On the basis of this finding and immunohistochemical analyses showing expression of cytokeratin 20 and lack of expression of cytokeratin 7 and the estrogen and progesterone receptors in SW626 cells, it is now thought that SW626 was more likely derived from a colorectal carcinoma that metastasized to the ovary rather than a primary ovarian carcinoma. Nevertheless, we believe that confusion about tumor type is an unlikely explanation for the *APC* mutations identified in our primary OEA (OE-32T), because we have verified that OE-32T is a well-differentiated endometrioid adenocarcinoma, arising in the setting of endometriosis. Furthermore, the tumor was confined to one ovary. Metastatic colon carcinomas typically involve both ovaries, and nearly half of all OEAs are associated with endometriosis (51).

A number of studies have shown that AXIN is critical for mediating the down-regulation of β-catenin [reviewed by Polakis (16)]. AXIN has binding sites for a number of proteins including GSK3β, β-catenin, APC, PP2Ac (the catalytic subunit of protein phosphatase 2A), and itself (26, 52). Although much remains to be learned about how AXIN functions, it appears to play a crucial role in facilitating the phosphorylation of β-catenin and APC by GSK3β (53). On the basis of its ability to down-regulate β-catenin and Wnt signaling, AXIN can be regarded as a tumor suppressor. Moreover, the somatic inactivating mutations found in *AXIN1* and *AXIN2* in some tumors are consistent with the tumor suppressor gene designation. Although biallelic inactivation of *AXIN1* and *AXIN2* has been identified in some human cancers, in others, one allele is mutant and the other is wild type (26, 27). Our findings, particularly when considered in light of data in the literature, offer support for the view that the *AXIN* mutations we report alter AXIN function and deregulate β-catenin. Akin to the mutations we describe in *CTNNB1* and *APC*, the mutations detected in *AXIN1* and *AXIN2* were clonal in nature. Two of the three mutant *AXIN* alleles described encode significantly truncated mutant proteins, removing defined functional domains in the AXIN1 and AXIN2 proteins. In the case of the missense mutant *AXIN1* allele identified in MDAH-2774 cells, we have presented clear evidence of nuclear accumulation of β-catenin (Fig. 2*D*) and increased TCF transcriptional activity (Fig. 1) in the tumor cells harboring the mutation. Although the TCF-dependent transcriptional activity in MDAH-2774 is modest compared with TOV-112D, our results are comparable with those of the Morin laboratory, which identified MDAH-2774 as one of four ovarian cancer cell lines with increased β-catenin/TCF transcriptional activity (34). The *AXIN1* sequence alteration in MDAH-2774, which affects a residue in the region of AXIN1 implicated in β-catenin binding, has not been identified in any of over 100 *AXIN1* alleles we and others have characterized thus far in other cancers (Refs. 25, 26 and data not shown). Collectively, these findings support the likely functional significance of the *AXIN1* defect in MDAH-2774, because these cells do not have mutations in *CTNNG1*, *CTNNB1*, *APC*, or *AXIN2*. Finally, we note that the primary tumor with the nonsense *AXIN1* mutation (OE-29T) did not show nuclear accumulation of β-catenin by immunohistochemistry. Given the limited number of tumors with *AXIN1* mutations identified to date, little is known about how well *AXIN1* mutation correlates with aberrant β-catenin localization. Even tumors with documented *CTNNB1* mutations do not invariably show readily recognizable nuclear or cytoplasmic accumulation of β-catenin protein (see for example, OE-14T and OE-19T; Table 2).

Previous functional studies of human tumor-derived mutants of *AXIN2* provide support for a dominant-negative effect of the mutant protein (27). Specifically, transfection of a cDNA encoding the same AXIN2 mutant as the one we identified in tumor OE-5T (2083, del G) into cells expressing wild-type AXIN2 resulted in activation of TCF-dependent transcription. Moreover, this mutant protein was found to be more stable than the wild-type AXIN2 protein in colon cancer cells. These findings are consistent with a dominant-negative role for the mutant AXIN2 protein.

In summary, we have shown here that β-catenin deregulation is a common feature of OEAs, and mechanisms for deregulating β-catenin include mutation of β-catenin itself and mutational inactivation of APC, AXIN1, and AXIN2. The defects in β-catenin regulation presumably lead to activated expression of TCF/LEF-regulated target genes. Although many of these remain unknown, a number of downstream targets have been proposed and include c-*MYC*, *CCND1/cyclin D1*, *PPARδ*, *gastrin*, *Cx43* (connexin 43), *WISP-1*, *WISP-2*, and *MMP-7* (matrilysin; Refs. 16, 18, 19, 21, 54–57). Downstream target genes of the β-catenin/TCF signaling pathway crucial for ovarian epithelial cell transformation will likely be up-regulated in all, or at least most, OEAs with β-catenin/TCF pathway defects. Further work evaluating gene expression patterns in OEAs with and without Wnt pathway defects will likely enrich our view of critical downstream TCF/LEF-regulated target genes.

REFERENCES

1. Greenlee, R. T., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics, 2000. CA Cancer J. Clin., *50:* 7–33, 2000.
2. Dubeau, L. The cell of origin of ovarian epithelial tumors and the ovarian surface epithelium dogma: does the emperor have no clothes? Gynecol. Oncol., *72:* 437–442, 1999.
3. Auersperg, N., Edelson, M. I., Mok, S. C., Johnson, S. W., and Hamilton, T. C. The biology of ovarian cancer. Semin. Oncol., *25:* 281–304, 1998.
4. Li, A. J., and Karlan, B. Y. Genetic factors in ovarian carcinoma. Curr. Oncol. Rep., *3:* 27–32, 2001.
5. Aunoble, B., Sanches, R., Didier, E., and Bignon, Y. J. Major oncogenes and tumor suppressor genes involved in epithelial ovarian cancer. Int. J. Oncol., *16:* 567–576, 2000.
6. Feeley, K. M., and Wells, M. Precursor lesions of ovarian epithelial malignancy. Histopathology, *38:* 87–95, 2001.
7. Obata, K., Morland, S. J., Watson, R. H., Hitchcock, A., Chenevix-Trench, G., Thomas, E. J., and Campbell, I. G. Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. Cancer Res., *58:* 2095–2097, 1998.
8. Fujita, M., Enomoto, T., Yoshino, K., Nomura, T., Buzard, G. S., Inoue, M., and Okudaira, Y. Microsatellite instability and alterations in the *hMSH2* gene in human ovarian cancer. Int. J. Cancer, *64:* 361–366, 1995.
9. King, B. L., Carcangiu, M. L., Carter, D., Kiechle, M., Pfisterer, J., Pfleiderer, A., and Kacinski, B. M. Microsatellite instability in ovarian neoplasms. Br. J. Cancer, *72:* 376–382, 1995.
10. Saegusa, M., and Okayasu, I. Frequent nuclear β-catenin accumulation and associated mutations in endometrioid-type endometrial and ovarian carcinomas with squamous differentiation. J. Pathol., *194:* 59–67, 2001.
11. Sagae, S., Kobayashi, K., Nishioka, Y., Sugimura, M., Ishioka, S., Nagata, M., Terasawa, K., Tokino, T., and Kudo, R. Mutational analysis of β-catenin gene in Japanese ovarian carcinomas: frequent mutations in endometrioid carcinomas. Jpn. J. Cancer Res., *90:* 510–515, 1999.
12. Palacios, J., and Gamallo, C. Mutations in the β-catenin gene (*CTNNB1*) in endometrioid ovarian carcinomas. Cancer Res., *58:* 1344–1347, 1998.
13. Gamallo, C., Palacios, J., Moreno, G., Calvo de Mora, J., Suarez, A., and Armas, A. β-Catenin expression pattern in stage I and II ovarian carcinomas: relationship with β-catenin gene mutations. Clinicopathological features, and clinical outcome. Am. J. Pathol., *155:* 527–536, 1999.
14. Wright, K., Wilson, P., Morland, S., Campbell, I., Walsh, M., Hurst, T., Ward, B., Cummings, M., and Chenevix-Trench, G. β-Catenin mutation and expression analysis in ovarian cancer: exon 3 mutations and nuclear translocation in 16% of endometrioid tumours. Int. J. Cancer, *82:* 625–629, 1999.
15. Cadigan, K. M., and Nusse, R. Wnt signaling: a common theme in animal development. Genes Dev., *11:* 3286–3305, 1997.
16. Polakis, P. Wnt signaling and cancer. Genes Dev., *14:* 1837–1851, 2000.
17. Peifer, M., and Polakis, P. Wnt signaling in oncogenesis and embryogenesis—a look outside the nucleus. Science (Wash. DC), *287:* 1606–1609, 2000.
18. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. Identification of c-MYC as a target of the APC pathway. Science (Wash. DC), *281:* 1509–1512, 1998.
19. Shtutman, M., Zhurinsky, J., Simcha, I., Albanese, C., D'Amico, M., Pestell, R., and Ben-Ze'ev, A. The *cyclin D1* gene is a target of the β-catenin/LEF-1 pathway. Proc. Natl. Acad. Sci. USA, *96:* 5522–5527, 1999.
20. Tetsu, O., and McCormick, F. β-Catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature (Lond.), *398:* 422–426, 1999.

21. Crawford, H. C., Fingleton, B. M., Rudolph-Owen, L. A., Goss, K. J., Rubinfeld, B., Polakis, P., and Matrisian, L. M. The metalloproteinase matrilysin is a target of β-catenin transactivation in intestinal tumors. Oncogene, *18:* 2883–2891, 1999.
22. Polakis, P. The oncogenic activation of β-catenin. Curr. Opin. Genet. Dev., *9:* 15–21, 1999.
23. Munemitsu, S., Albert, I., Souza, B., and Polakis, P. Regulation of intracellular β-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc. Natl. Acad. Sci. USA, *92:* 3046–3050, 1995.
24. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. Activation of β-catenin-TCF signaling in colon cancer by mutations in β-catenin or APC. Science (Wash. DC), *275:* 1787–1790, 1997.
25. Webster, M. T., Rozycka, M., Sara, E., Davis, E., Smalley, M., Young, N., Dale, T. C., and Wooster, R. Sequence variants of the *AXIN* gene in breast, colon, and other cancers: an analysis of mutations that interfere with GSK3 binding. Genes Chromosomes Cancer, *28:* 443–453, 2000.
26. Satoh, S., Daigo, Y., Furukawa, Y., Kato, T., Miwa, N., Nishiwaki, T., Kawasoe, T., Ishiguro, H., Fujita, M., Tokino, T., Sasaki, Y., Imaoka, S., Murata, M., Shimano, T., Yamaoka, Y., and Nakamura, Y. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. Nat. Genet., *24:* 245–250, 2000.
27. Liu, W., Dong, X., Mai, M., Seelan, R. S., Taniguchi, K., Krishnadath, K. K., Halling, K. C., Cunningham, J. M., Qian, C., Christensen, E., Roche, P. C., Smith, D. I., and Thibodeau, S. N. Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating β-catenin/TCF signalling. Nat. Genet., *26:* 146–147, 2000.
28. Caca, K., Kolligs, F. T., Ji, X., Hayes, M., Qian, J., Yahanda, A., Rimm, D. L., Costa, J., and Fearon, E. R. β- and γ-catenin mutations, but not E-cadherin inactivation, underlie T-cell factor/lymphoid enhancer factor transcriptional deregulation in gastric and pancreatic cancer. Cell Growth Differ., *10:* 369–376, 1999.
29. Behrens, J. Cadherins and catenins: role in signal transduction and tumor progression. Cancer Metastasis Rev., *18:* 15–30, 1999.
30. Zhurinsky, J., Shtutman, M., and Ben-Ze'ev, A. Plakoglobin and β-catenin: protein interactions, regulation and biological roles. J. Cell Sci., *113:* 3127–3139, 2000.
31. Allan, G. J., Cottrell, S., Trowsdale, J., and Foulkes, W. D. Loss of heterozygosity on chromosome 5 in sporadic ovarian carcinoma is a late event and is not associated with mutations in APC at 5q21–22. Hum. Mutat., *3:* 283–291, 1994.
32. Kolligs, F. T., Kolligs, B., Hajra, K. M., Hu, G., Tani, M., Cho, K. R., and Fearon, E. R. γ-Catenin is regulated by the APC tumor suppressor and its oncogenic activity is distinct from that of β-catenin. Genes Dev., *14:* 1319–1331, 2000.
33. Russell, P. Surface epithelial-stromal tumors of the ovary. *In:* R. J. Kurman (ed.), Blaustein's Pathology of the Female Genital Tract, Ed. 4, pp. 705–782. New York: Springer-Verlag, 1994.
34. Furlong, M. T., and Morin, P. J. Rare activation of the TCF/β-catenin pathway in ovarian cancer. Gynecol. Oncol., *77:* 97–104, 2000.
35. Tago, K., Nakamura, T., Nishita, M., Hyodo, J., Nagai, S., Murata, Y., Adachi, S., Ohwada, S., Morishita, Y., Shibuya, H., and Akiyama, T. Inhibition of Wnt signaling by ICAT, a novel β-catenin-interacting protein. Genes Dev., *14:* 1741–1749, 2000.
36. Hart, M. J., de los Santos, R., Albert, I. N., Rubinfeld, B., and Polakis, P. Downregulation of β-catenin by human AXIN and its association with the APC tumor suppressor, β-catenin and GSK3β. Curr. Biol., *8:* 573–581, 1998.
37. Lin, Y. M., Kato, T., Satoh, S., Nakamura, Y., and Furukawa, Y. Identification of novel polymorphisms in the *AXIN1* and *CDX-2* genes. J. Hum. Genet., *45:* 254–256, 2000.
38. Wu, R., Connolly, D. C., Ren, X., Fearon, E. R., and Cho, K. R. Somatic mutations of the *PPP2R1B* candidate tumor suppressor gene at chromosome 11q23 are infrequent in ovarian carcinomas. Neoplasia, *1:* 311–314, 1999.
39. Ilyas, M., Tomlinson, I. P., Rowan, A., Pignatelli, M., and Bodmer, W. F. β-Catenin mutations in cell lines established from human colorectal cancers. Proc. Natl. Acad. Sci. USA, *94:* 10330–10334, 1997.
40. Rubinfeld, B., Robbins, P. D., El-Gamil, M., Albert, I., Porfiri, E., and Polakis, P. Stabilization of β-catenin by genetic defects in melanoma cell lines. Science (Wash. DC), *275:* 1790–1792, 1997.
41. Koch, A., Denkhaus, D., Albrecht, S., Leuschner, I., von Schweinitz, D., and Pietsch, T. Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the β-catenin gene. Cancer Res., *59:* 269–273, 1999.
42. Zurawel, R. H., Chiappa, S. A., Allen, C., and Raffel, C. Sporadic medulloblastomas contain oncogenic β-catenin mutations. Cancer Res., *58:* 896–899, 1998.
43. Voeller, H. J., Truica, C. I., and Gelmann, E. P. β-Catenin mutations in human prostate cancer. Cancer Res., *58:* 2520–2523, 1998.
44. Chesire, D. R., Ewing, C. M., Sauvageot, J., Bova, G. S., and Isaacs, W. B. Detection and analysis of β-catenin mutations in prostate cancer. Prostate, *45:* 323–334, 2000.
45. Fukuchi, T., Sakamoto, M., Tsuda, H., Maruyama, K., Nozawa, S., and Hirohashi, S. β-Catenin mutation in carcinoma of the uterine endometrium. Cancer Res., *58:* 3526–3528, 1998.
46. Mirabelli-Primdahl, L., Gryfe, R., Kim, H., Millar, A., Luceri, C., Dale, D., Holowaty, E., Bapat, B., Gallinger, S., and Redston, M. β-Catenin mutations are specific for colorectal carcinomas with microsatellite instability but occur in endometrial carcinomas irrespective of mutator pathway. Cancer Res., *59:* 3346–3351, 1999.
47. Saegusa, M., Hashimura, M., Yoshida, T., and Okayasu, I. β-Catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. Br. J. Cancer, *84:* 209–217, 2001.
48. Schlosshauer, P. W., Pirog, E. C., Levine, R. L., and Hedrick-Ellenson, L. Mutational analysis of the *CTNNB1* and *APC* genes in uterine endometrioid carcinoma. Mod. Pathol., *13:* 1066–1071, 2000.
49. Scully, R. E., Young, R. H., and Clement, P. B. Tumors of the Ovary, Maldeveloped Gonads, Fallopian Tube, and Broad Ligament. Atlas of Tumor Pathology, Third Series, Fascicle, Vol. 23. Washington, DC: Armed Forces Institute of Pathology, 1998.
50. Furlong, M. T., Hough, C. D., Sherman-Baust, C. A., Pizer, E. S., and Morin, P. J. Evidence for the colonic origin of ovarian cancer cell line SW626. J. Natl. Cancer Inst., *91:* 1327–1328, 1999.
51. Fukunaga, M., Nomura, K., Ishikawa, E., and Ushigome, S. Ovarian atypical endometriosis: its close association with malignant epithelial tumours. Histopathology, *30:* 249–255, 1997.
52. Smalley, M. J., Sara, E., Paterson, H., Naylor, S., Cook, D., Jayatilake, H., Fryer, L. G., Hutchinson, L., Fry, M. J., and Dale, T. C. Interaction of AXIN and Dvl-2 proteins regulates Dvl-2-stimulated TCF-dependent transcription. EMBO J., *18:* 2823–2835, 1999.
53. Ikeda, S., Kishida, M., Matsuura, Y., Usui, H., and Kikuchi, A. GSK-3β-dependent phosphorylation of adenomatous polyposis coli gene product can be modulated by β-catenin and protein phosphatase 2A complexed with AXIN. Oncogene, *19:* 537–545, 2000.
54. He, T. C., Chan, T. A., Vogelstein, B., and Kinzler, K. W. PPARδ is an APC-regulated target of nonsteroidal anti-inflammatory drugs. Cell, *99:* 335–345, 1999.
55. Koh, T. J., Bulitta, C. J., Fleming, J. V., Dockray, G. J., Varro, A., and Wang, T. C. Gastrin is a target of the β-catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. J. Clin. Investig., *106:* 533–539, 2000.
56. van der Heyden, M. A., Rook, M. B., Hermans, M. M., Rijksen, G., Boonstra, J., Defize, L. H., and Destree, O. H. Identification of connexin43 as a functional target for Wnt signalling. J. Cell Sci., *111:* 1741–1749, 1998.
57. Pennica, D., Swanson, T. A., Welsh, J. W., Roy, M. A., Lawrence, D. A., Lee, J., Brush, J., Taneyhill, L. A., Deuel, B., Lew, M., Watanabe, C., Cohen, R. L., Melhem, M. F., Finley, G. G., Quirke, P., Goddard, A. D., Hillan, K. J., Gurney, A. L., Botstein, D., and Levine, A. J. *WISP* genes are members of the connective tissue growth factor family that are up-regulated in Wnt-1-transformed cells and aberrantly expressed in human colon tumors. Proc. Natl. Acad. Sci. USA, *95:* 14717–14722, 1998.

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