β-Catenin Expression Pattern in Stage I and II Ovarian Carcinomas

Relationship with β-Catenin Gene Mutations, Clinicopathological Features, and Clinical Outcome

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The immunohistochemical expression pattern of β-catenin has been correlated with β-catenin gene mutations, clinicopathological features, and disease outcome in 69 stage I and II ovarian carcinomas. β-Catenin expression was localized in the nuclei, in addition to the cytoplasm and membrane, in 11 tumors (16%): nine endometrioid carcinomas with widespread nuclear expression and two serous carcinomas with focal nuclear expression. The remaining 58 carcinomas (84%) only had membranous β-catenin expression. All but one of the endometrioid carcinomas with nuclear β-catenin expression had considerable squamous metaplasia, and five of these cases had large areas of endometrioid tumor of low malignant potential. In addition, β-catenin nuclear expression was observed in atypical epithelial cells in endometriotic glands adjacent to an endometrioid carcinoma. Sequencing was performed on 25 tumors and corresponding normal tissue: all 13 endometrioid tumors as well as 12 carcinomas of other histological types (four serous, two clear cell, two mucinous, and two mixed). There were oncogenic mutations in the phosphorylation sequence for GSK-3β in exon 3 of the β-catenin gene in seven endometrioid carcinomas with β-catenin nuclear expression. Three mutations affected codon 32 (D32G, D32Y, and D32Y), one affected codon 33 (S33C), two affected codon 37 (S37C and S37F), and one affected codon 41 (T41A). No mutations were observed in the other 18 carcinomas analyzed, comprising two endometrioid and two serous carcinomas with β-catenin nuclear expression, and 14 carcinomas of different histological types with only membranous expression. In the univariate and multivariate survival analyses, β-catenin nuclear expression was selected as an indi-cator of good prognosis, because no patient whose tumor expressed β-catenin in the nuclei showed relapses or died, in contrast to the 19 relapses and deaths among patients with tumors that only had β-catenin membranous expression, including three of the four patients with endometrioid carcinomas. Oncogenic β-catenin mutation is characteristic of a group of endometrioid carcinomas with a good prognosis, most of which originate from previous benign or borderline lesions. Endometrioid carcinomas with exclusively membranous expression of β-catenin seem to represent a different subgroup of carcinomas that probably have a worse prognosis. In early-stage ovarian cancer, determination of the β-catenin expression pattern could prove to be a useful marker for selecting low-risk patients. *(Am J Pathol 1999, 155:527–536)*

Ovarian cancer is a highly agressive gynecological malignancy affecting approximately 27,000 women per year in the United Sates and producing almost 15,000 deaths yearly.[1] Although the prognosis of early-stage ovarian cancer is considerably better than in advanced disease, 30–40% of early-stage patients will eventually die as a result of their tumors.[2] High-grade histology, tumor cells in ascites or peritoneal washings, a ruptured capsule, or the presence of a tumor on the external surface have been described as unfavorable prognostic factors in early-stage ovarian cancer,[3,4] and adjuvant chemotherapy is strongly recommended.[2]

Relatively little is known about the molecular events that lead to the development of ovarian cancer, and no molecular markers are generally accepted as prognostic indicators. Although most cases of epithelial tumors of

the ovary seem to have a common origin in the surface epithelium covering the ovary, different pathways and genetic alterations have been implicated in the development of cystadenomas, borderline tumors, and carcinomas of different histological subtypes and stages of progression. For example, loss of heterozygocity and a complete loss of chromosome 17 is characteristic of the advanced stages of serous carcinomas,[5] whereas ras mutation participates in the development of tumors with mucinous differentiation, but not in other histological types.[6] Recently, we have reported data that strongly suggest that β-catenin may function as an oncogen in the initiation of some ovarian endometrioid carcinomas in which β-catenin is mainly expressed in the nucleus and cytoplasm.[7] However, the clinical implications of this finding remain to be established.

β-Catenin is a multifunctional protein involved in at least two important biological processes: cell-cell adhesion and signal transduction (transcriptional activation).[8] The role of β-catenin in adhesion is well established. In the adherens junctions of epithelial cells, the cytoplasmic domain of E-cadherin organizes a peripheral protein complex, including α-catenin, β-catenin, and γ-catenin (plakoglobin), that is necessary for adhesion to occur.[9] The role of β-catenin in the linkage between α-catenin and E-cadherin in this complex is probably regulated by epidermal growth factor-mediated tyrosine phosphorylation of the β-catenin.[8,9] Molecules of the cadherin/catenin complex have been implicated in differentiation and tumor progression, because their reduced expression is not only frequent in poorly differentiated tumors, but also might produce loss of adhesiveness and increased invasive and metastatic potential.[10–13]

In addition, β-catenin participates in the transduction of signals and activates transcription by forming complexes with DNA binding proteins in the T-cell factor-lymphoid enhancer factor (TcF-lef) family.[14] The free (cytoplasmic) β-catenin level is low in normal cells because the protein is targeted for destruction in the ubiquitin-proteasome system[15] by adenomatous polyposis coli (APC) protein together with glycogen synthetase kinase-3β (GSK-3β)[16] and other molecules such as axin or conductin[8]; thus wild-type APC plays an essential role in the clearance of unnecessary β-catenin from the cytoplasm. Disruption of the APC-mediated regulation of the β-catenin-Tcf pathway was first implicated in the development of colon carcinomas and melanoma.[17,18] Mutations that inactivate APC increase cytoplasmic levels of free β-catenin,[19] which may then act as an oncoprotein through constitutive β-catenin-Tcf-regulated transcription.[14] In the same manner, activating β-catenin mutations render this pathway insensitive to the effect of WT APC.[17,18] To date, oncogenic β-catenin mutations have been described in several malignancies, such as carcinomas of the colon,[17,20–23] ovary,[7] prostate,[24] liver,[25] and endometrium[26] and medulloblastomas.[27]

This study analyzes the expression pattern of β-catenin in a series of 69 stage I and II ovarian carcinomas and their relationships with β-catenin gene mutation, clinicopathological features, and survival to better understand the biological and clinical relevance of β-catenin pathway activation in ovarian cancer. Some of the clinicopathological and genetic features of 30 cases have previously been reported.[7]

Materials and Methods

Patients

This study was conducted on 69 primary ovarian carcinomas diagnosed at the Department of Pathology of La Paz Hospital, Madrid. All cases were in stage I or II and had a minimum follow-up period of 5 years. Staging was done following the International Federation of Gynecology and Obstetrics (FIGO) system. Retrospective reviews of patient files were performed to obtain all pertinent data on the primary tumor, type of surgery, adjuvant treatment, recurrence, and survival. If necessary, patients were retrospectively assigned a FIGO stage on the basis of their clinical history. The tumors were routinely fixed in 10% formalin and embedded in paraffin. Histological typing was performed according to the World Health Organization classification. Histological, immunohistochemical, and DNA studies were performed on formalin-fixed, paraffin-embedded tissue samples.

Immunohistochemistry

Immunohistochemistry for β-catenin was performed by the avidin-biotin-alkaline phosphatase method, as previously reported.[7] A heat-induced antigen retrieval step (deparaffinized sections were immersed in 0.01 mol/L sodium citrate buffer (pH 6.0) and incubated in a pressure cooker for 3 minutes) was performed. The mouse anti-human β-catenin monoclonal antibody (Transduction Laboratories, Lexington, KY) was applied at a dilution of 1:200. In negative controls, the primary antibody was omitted or replaced with an irrelevant antibody.

Evaluation of Immunohistochemical Staining

Two patterns of β-catenin expression were considered: membranous, if β-catenin was localized in the cell membrane only; and nuclear, when β-catenin was expressed in the nucleus, irrespective of the percentage of stained nuclei or simultaneous expression of β-catenin in membrane and cytoplasm.

A semiquantitative estimation of membranous β-catenin expression was made, using a composite score obtained by adding the values of the immunoreaction intensity and relative abundance of the β-catenin immunoreactive cells, as previously reported for cadherins.[28,29] Briefly, the intensity was graded from 0 (equivalent to background staining of the acellular stroma) to +3 (intense stain equivalent to normal surface epithelium). The abundance of β-catenin-positive cells was graded from 0 to 4 (0 = less than 5% of positive cells; 1 = 5–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%). β-Catenin expression was considered preserved when the composite score was 6 or 7. Cases with

scores between 0 and 5 were considered to be tumors with reduced β-catenin expression.

DNA Analysis

Polymerase chain reaction (PCR) was performed as previously reported[7] on 25 tumors and corresponding normal tissue: all 13 endometrioid tumors as well as 12 carcinomas of other histological types (four serous, two clear cell, two mucinous, and two mixed). DNA was extracted from paraffin blocks containing a large proportion of epithelial tumor cells (>75%), but no microdissection of selected areas was performed. Normal tissue in each case consisted of samples from fallopian tube or myometrium.

The primers used were 240F (ATG GAA CCA GAC AGA AAA GC) and 439F (GCT ACT TGT TCT GAG TGA AG), which amplified a 200-bp fragment of exon 3 of the β-catenin gene, encompassing the sequence for GSK-3β phosphorylation. After purification, PCR products were sequenced in a 373 Automated Sequencer or a 310 Automater Sequencer (Applied Biosystems, Foster City, CA), using the Abi Prism dRodhamine Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). PCR primers were used as the primers for sequencing the two strands of the amplified products. Cases with abnomal DNA sequences were subjected to DNA extraction from additional tissue sections, PCR, and sequencing, to confirm the presence of true mutations.

Statistical Methods

The χ^2 test was used to analyze the statistical significance of the relationship between β-catenin expression and the clinicopathological and immunohistochemical variables. Relapse-free survival and overall survival, defined as the time from diagnosis to relapse or death, respectively, were used as a measure of prognosis. Univariate survival curves were estimated using the Kaplan and Meier method and compared using the log rank test. Multivariate analysis was done using Cox's proportional hazards regression model. Analyses were carried out using JMP (Version 3.0.1; SAS Institute, Cary, NC).

Results

Clinicopathological Features

Some clinicopathological, inmunohistochemical, and genetic features of 30 cases have previously been reported.[7] The mean age of the 69 patients was 55 years (range 19–77). There were 19 (27.5%) stage Ia, 4 (6%) stage Ib, 28 (40.5%) stage Ic, 5 (7%) stage IIa, 9 (13%) stage IIb, and 4 (6%) stage IIc tumors. There were 27 (39%) grade I carcinomas, 28 (41%) grade II, and 14 (20%) grade III. No patient showed macroscopic residual disease after surgery. All patients received postoperative therapy: 17 (24.6%) radiotherapy and 52 (75.4%) chemotherapy: 33 melphalan and adryamicine, 15 melphalan alone, and 4 cisplatin-based chemotherapy.

β-Catenin Expression and Correlations with Clinicopathological Features

All analyzed tumors showed membrane β-catenin expression with variable extension and intensity, but simultaneous nuclear β-catenin expression (nuclear pattern) was observed in only 11 (16%) ovarian carcinomas (Figure 1A); the remaining 58 tumors (84%) only had membrane expression (membranous pattern) (Figure 1B). The expression pattern (nuclear *versus* membranous) correlated significantly with the histological type (Table 1), inasmuch as 9 cases with nuclear expression were endometrioid carcinomas. Nuclear expression was also observed in two serous carcinomas. In endometrioid lesions, the percentage of stained nuclei ranged between 25% and 90% of the neoplastic cells. In contrast, in serous carcinomas the percentage of immunoreactive nuclei did not exceed 5%. The different β-catenin expression patterns did not correlate with tumor grade and stage. In addition, postoperative therapy was similar in tumors with a nuclear or membranous β-catenin expression pattern (Table 1).

All but one endometrioid carcinoma with nuclear β-catenin expression also had evident squamous metaplasia, and five of these cases had large areas of endometrioid tumor of low malignant potential with an adenofibromatous, papillary, or mixed growth pattern (Table 2). Endometrioid carcinomas with β-catenin membranous expression only did not have squamous metaplasia, and two cases had a growth pattern resembling that of sexcord stromal tumors. Four mixed carcinomas with an endometrioid component showed only β-catenin membranous expression. Nuclear β-catenin expression was observed in epithelial cells of endometriotic cyst (Figure 1C) adjacent to an endometrioid carcinoma with β-catenin nuclear expression.

As previously noted, β-catenin expression was observed in the membrane in all of the 69 carcinomas, but only 15 cases (22%) had preserved membrane expression (score 6–7), and the remaining 54 carcinomas had reduced membrane expression (score 3–5). The status of membrane β-catenin expression (preserved *versus* reduced) did not correlate to the histological type, grade, or stage of the patient's tumor (Table 3).

β-Catenin Gene Mutations

Direct sequence analysis of the PCR amplification products showed heterozygous substitution mutations of β-catenin gene exon 3 in seven endometrioid carcinomas with nuclear β-catenin expression: three at codon 32 (Figure 1E and 1F), one at codon 33 (Figure 1F), two at codon 37, and one at codon 41. At codon 32, two GAC to TAC (D32Y) changes and a GAC to GGC (D32G) change were observed; at codon 33, a TCT to TGT change (S33C) was observed; at codon 37, a TCT to TTT change (S37F) and a TCT to TGT change (S37C) were observed; and finally, an ACC to GCC change at codon 41 (T41A) was observed. To further confirm sequencing data in the cases with mutation in S37, PCR products were digested
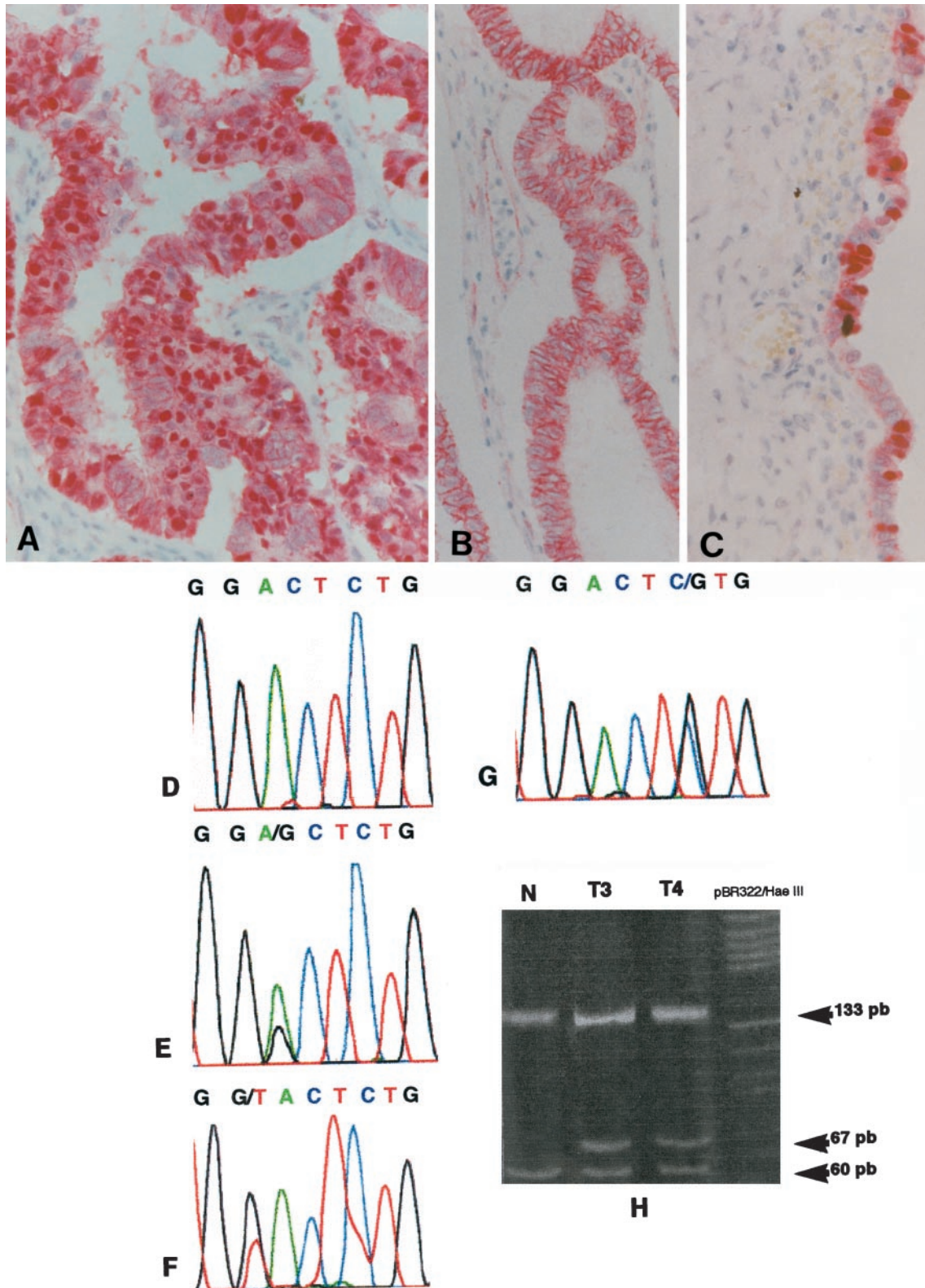
Figure 1. A: Nuclear β-catenin expression pattern in an endometrioid carcinoma. **B**: Membranous β-catenin expression pattern in an endometrioid carcinoma. **C**: Nuclear β-catenin expression in some nuclei of epithelial cells of an endometriotic cyst. **D**: Normal sequence of codons 32 and 33 in exon 3 of β-catenin gene. **E**: GAC to GGC change in codon 32 (D32G) in tumor 1. **F**: GAC to TAC change in codon 32 (D32Y) in tumor 3. **G**: TCT to TGT change in codon 33 (S33C) in tumor 4. **H**: Analysis with *Hinf*I restriction endonuclease of PCR products from cases 3 and 4. In normal tissue (N) digestion produces three fragments of 133, 60, and 7 bp. In tumor tissues, heterozygous mutations in codons 32 and 33 eliminate a restriction site in the mutated allele and generate an additional fragment of 67 bp.

Table 1. Relationships between β-Catenin Expression and Clinicopathological Features in Stage I and II Ovarian Carcinomas*

	Nuclear β-catenin† expression pattern	Membrane β-catenin expression pattern	P value
Histological type			
Endometrioid (n = 13)	9 (70%)	4 (30%)	
Serous (n = 16)	2 (12.5%)	14 (87.5)	
Mucinous (n = 13)	0	13 (100%)	
Clear cell (n = 18)	0	18 (100%)	
Mixed (n = 9)	0	9 (100%)	0.000
Grade			
I (n = 27)	5 (18.5%)	22 (81.5%)	
II (n = 28)	5 (18%)	23 (82%)	
III (n = 14)	1 (7.2%)	13 (96.8%)	0.552
FIGO stage			
Ia-Ib (n = 23)	5 (21.7%)	18 (78.3%)	
Ic-II (n = 46)	6 (15.2%)	40 (84.8%)	0.36
Postoperative therapy			
Adriamycine and melphalan (n = 33)	5 (15.2%)	28 (84.8%)	
Melphalan (n = 15)	2 (13.4%)	13 (86.6%)	
Cisplatinun (n = 4)	0	4 (100%)	
Radiotherapy (n = 17)	4 (23.5%)	13 (76.5%)	0.663

*χ^2 test.
†Nuclear β-catenin expression pattern: When the protein was expressed in the nucleus, irrespective of the percentage of stained nuclei or simultaneous expression of β-catenin in membrane and cytoplasm; membranous β-catenin expression pattern: if β-catenin was localized in the cell membrane only.

with *Xmn*I restriction endonuclease. Mutations affecting codons 32 and 33 were confirmed by *Hinf*I restriction analysis (Figure 1H). All detected mutations were also confirmed by resequencing.

No β-catenin gene mutations were observed in two endometrioid carcinomas with nuclear β-catenin expression pattern, four endometrioid carcinomas with a membranous β-catenin pattern (Table 3), or 12 non-endometrioid carcinomas, two serous carcinomas with focal nuclear β-catenin expression, and 10 carcinomas with membranous β-catenin pattern.

Outcome Analysis

The mean follow-up time of the study was 8.9 ± 6.8 years (range 1–24 years; median follow-up period 7 years). Among the 69 patients, 19 (33.3%) showed a relapse, which occurred between 4 and 46 months after surgery. All patients who relapsed subsequently died (13–48 months after surgery) of the disease.

The variables analyzed were the type of postoperative therapy (radiotherapy, melphalan, melphalan and adryamicine, and cisplatinun- based chemotherapy), histological type, histological grade, stage (Ia-b *versus* Ic-II), β-catenin expression pattern (nuclear *versus* membranous), and membrane β-catenin expression (preserved *versus* reduced).

Relapse-free survival was significantly related to histological grade ($\chi^2 = 26.61$; $P = 0.0000$), stage ($\chi^2 = 9.61$; $P = 0.0019$), and β-catenin expression pattern ($\chi^2 = 5.82$; $P = 0.016$), but was unrelated to the type of postoperative therapy ($\chi^2 = 5.11$; $P = 0.163$), membrane β-catenin expression ($\chi^2 = 1.93$; $P = 0.164$), and histological type ($\chi^2 = 6.88$; $P = 0.153$) in the univariate

Table 2. Clinicopathological, Immunohistochemical, and Genetic Characteristics of 13 Endometrioid Carcinomas

Case	Age	Stage	Grade	Endometriosis	ETLMP	Squamous metaplasia	β-Catenin pattern	Expression membrane	Mutated codon	Base change	Amino acid change	Follow-up (years)
1	61	1c	1	Absent	Present	Present	Nuclear	Reduced	32	GAC to GGC	Asp to Gly	NED 6
2	49	1b	1	Absent	Present	Present	Nuclear	Preserved	32	GAC to TAC	Asp to Tyr	NED 6
3	47	1b	2	Absent	Absent	Present	Nuclear	Reduced	32	GAC to TAC	Asp to Tyr	NED 16
4	50	2b	2	Absent	Absent	Present	Nuclear	Reduced	33	TCT to TGT	Ser to Cys	NED 11
5	48	1a	1	Absent	Present	Present	Nuclear	Preserved	37	TCT to TGT	Ser to Cys	NED 8
6	64	2a	1	Present	Present	Present	Nuclear	Preserved	37	TCT to TTT	Ser to Phe	NED 11
7	50	2a	2	Present	Absent	Present	Nuclear	Preserved	41	ACC to GCC	Thr to Ala	NED 12
8	38	1c	2	Present	Present	Present	Nuclear	Reduced	NS			NED 22
9	58	1c	3	Absent	Absent	Absent	Nuclear	Reduced	NS			NED 14
10	66	2c	2	Present	Absent	Absent	Membranous	Reduced	NS			NED 6
11	49	1c	2	Absent	Absent	Absent	Membranous	Reduced	NS			DD 2
12	61	2c	2	Absent	Absent	Absent	Membranous	Reduced	NS			DD 2
13	27	1a	3	Absent	Absent	Absent	Membranous	Reduced	NS			DD 3

Endometriosis in case 10 affected the contralateral ovary, whereas the tumorous ovary was affected in cases 6, 7, and 8. This last case arose directly from a endometriotic cyst. ETLMP: Endometrioid tumor of low malignant potential. NS: normal sequence. NED: No evidence of disease. DD: Died of disease.

Table 3.   Relationships between β-Catenin Membrane Expression and Clinicopathological Features in Stage I and II Ovarian Carcinomas*

	Preserved β-catenin expression pattern (score 6–7)	Reduced β-catenin membrane expression (score 0–5)	P value
Histological type			
Endometrioid ($n = 13$)	5 (38%)	8 (62%)	
Serous ($n = 16$)	1 (6%)	15 (94%)	
Mucinous ($n = 23$)	3 (23%)	20 (77%)	
Clear cell ($n = 18$)	5 (28%)	13 (72%)	
Mixed ($n = 9$)	1 (11%)	8 (89%)	0.210
Grade			
I ($n = 27$)	8 (30%)	19 (70%)	
II ($n = 28$)	5 (17%)	23 (83%)	
III ($n = 14$)	2 (14%)	12 (86%)	0.431
FIGO stage			
Ia-Ib ($n = 23$)	5 (22%)	18 (78%)	
Ic-II ($n = 46$)	10 (22%)	36 (78%)	1

*χ^2 test.

analysis. Multivariate analysis also selected histological grade ($\chi^2 = 16.82$; $P = 0.0022$; risk ratio = 4.63; 95% confidence interval = 1.40–20.82), stage ($\chi^2 = 7.96$; $P = 0.0048$; risk ratio = 5.81; 95% confidence interval = 1.61–37.18), and β-catenin pattern ($\chi^2 = 1028$; $P = 0.003$; risk ratio = 0.000005) as independent predictors of relapse-free survival.

Overall survival was also significantly related to histological grade ($\chi^2 = 21.12$; $P = 0.0000$), stage ($\chi^2 = 9.92$; $P = 0.0016$), and β-catenin expression pattern ($\chi^2 = 6.74$; $P = 0.0094$), but was unrelated to the type of postoperative therapy ($\chi^2 = 3.39$; $P = 0.334$), membrane β-catenin expression ($\chi^2 = 1.38$; $P = 0.51$), and histological type ($\chi^2 = 6.37$; $P = 0.17$) in the univariate analysis. Multivariate analysis also selected histological grade ($\chi^2 = 12.25$; $P = 0.0022$; risk ratio = 4.13; 95% confidence interval = 1.25–18.54), stage ($\chi^2 = 7.54$; $P = 0.0060$; risk ratio = 5.54; 95% confidence interval = 1.54–35.41), and β-catenin pattern ($\chi^2 = 8.91$; $P = 0.0028$; risk ratio = 0.000005), as independent predictors of OS. The nuclear pattern of β-catenin expression was an indicator of good prognosis, inasmuch as not one recurrence or death occurred among these patients.

Discussion

This study confirms our previous observation[7] in a reduced number of cases that β-catenin expression in ovarian cancer has two patterns: nucleocytoplasmic and membranous, which reflect the two main known functions of β-catenin: signal transduction and cell adhesion. Widespread β-catenin nuclear expression is mainly observed in endometrioid carcinoma, suggesting that activation of the β-catenin-Tcf signaling pathway mediates development in a group of cases of this subtype of ovarian cancer. Although the activation of this signaling pathway could hypothetically be achieved by different molecular events, this study demonstrated that most ovarian endometrioid carcinomas with nucleocytoplasmic expression of β-catenin have β-catenin-gene mutations.

Ovarian carcinomas may arise *de novo* from surface ovarian epithelium (or epithelial inclusion cysts) or from benign or borderline lesions.[30] It has been suggested that most serous carcinomas arise *de novo*, whereas most mucinous carcinomas originate from benign or borderline tumors.[31] A proportion of endometrioid carcinomas may arise from previous benign lesions such as endometriosis and benign tumors of the same histological type.[32,33] Endometrioid tumors of the ovary are either benign, usually taking the form of an adenofibroma or cystoadenofibroma, or overtly malignant. Between these two well-established categories, there is a spectrum of lesions that have received different names in the literature (proliferating endometrioid adenofibromas,[32] atypical and borderline endometriod adenofibromas,[33] proliferative endometrioid adenofibromas, and endometrioid tumors of low malignant potential[34,35]). Proliferating, borderline, or low-malignant-potential endometrioid tumors are characterized by adenocystadenofibromatous or papillary growth, epithelial proliferation, and atypia without stromal invasion.[32–35] In addition, they frequently show squamous metaplasia and have an excellent prognosis, although carcinomas may arise in them.[32–35] We previously reported β-catenin mutation in one borderline or low-malignant-potential endometrioid tumor. In this study we observed β-catenin nuclear expression in five carcinomas with large areas of endometrioid tumor of low malignant potential, four of which had β-catenin mutations. Although we have not studied benign endometrioid adenofibromas, these data indicate that β-catenin mutation would be implicated in the transformation process leading to the development of a subgroup of endometrioid carcinomas that have borderline or low-malignant-potential endometrioid tumors as their precursor. In addition, the observation of β-catenin nuclear expression in endometriotic glands adjacent to one carcinoma with β-catenin gene mutation suggests that β-catenin could be implicated in the malignant transformation of some cases of endometriosis.

We also observed β-catenin mutations in three endometrioid carcinomas without associated preinvasive areas but with evident squamous metaplasia. In contrast, none of the four endometrioid tumors with only membranous expression of β-catenin and without β-catenin mutations had squamous metaplasia. This finding may indicate that β-catenin mutations could be implicated in the genesis of so-called adenoacanthomas, a suggestion that must be confirmed in a larger series of cases.

Since the original description of oncogenic β-catenin mutation, several tumor types have been analyzed. β-Catenin point mutations have been reported in 2%, 5%, 13%, and 19% of medulloblastomas,[27] prostate carcinomas,[24] endometrial carcinomas,[26] and hepatocellular carcinomas,[25] respectively. Candidus et al[36] have not found evidence for mutation in this gene in gastric (diffuse and intestinal subtypes) and breast (ductal and lobular subtypes) carcinomas, although their sample was small. In ovarian carcinomas, we have observed β-catenin mutations in only 10% of all stage I and II tumors analyzed, but in 50% of the endometrioid carcinomas. This is the highest frequency of β-catenin mutation so far reported in any histological type of human carcinoma and is probably due to the characteristics of the sample analyzed (low stages and a high proportion of cases originated in preinvasive lesions).

Most studies on β-catenin mutations have only studied the consensus sequence for GSK-3β phosphorylation in exon 3. Mutations affecting this sequence, mainly in the amino acids implicated in the down-regulation of β-catenin through phosphorylation by this serine/threonine kinase (Ser,[33] Ser,[37] Thr[41], and Ser[45]),[17] probably render a fraction of cellular β-catenin insensitive to APC-mediated down-regulation and are responsible for the up-regulation of cytoplasmic β-catenin and its distribution in the nuclei of tumor cells, as was immunohistochemically detected in the present series. Mutation at codon 32 changing a highly conserved aspartic acid was observed in three ovarian carcinomas. Mutations have been reported at this codon in hepatocellular[25] and prostate[25] carcinomas, and it has been suggested that the alteration may change the protein structure and inhibit phosphorylation.

Although all reported β-catenin point mutations in human carcinoma samples affect the sequence for GSK-3β phosphorylation, the frequency of mutations at each codon and the type of mutation differ by tumor type, probably indicating different etiologies (Figure 2). For example, in hepatocellular carcinoma, but not in other tumor types, the change from G to A or T predominates.[25] These types of β-catenin mutations have been reported in colon carcinomas induced by azoxymethane[37] and heterocyclic amines,[38] which have a preference for forming DNA adducts at guanines. In endometrial carcinoma, 50% of the mutations are C to T changes, suggesting the action of carcinogens that induce deamination of DNA bases.[26]

We did not find β-catenin gene mutations in two endometrioid carcinomas with nuclear and cytoplasmic β-catenin expression; however, our DNA analysis was very limited, and genetic alterations other than mutations
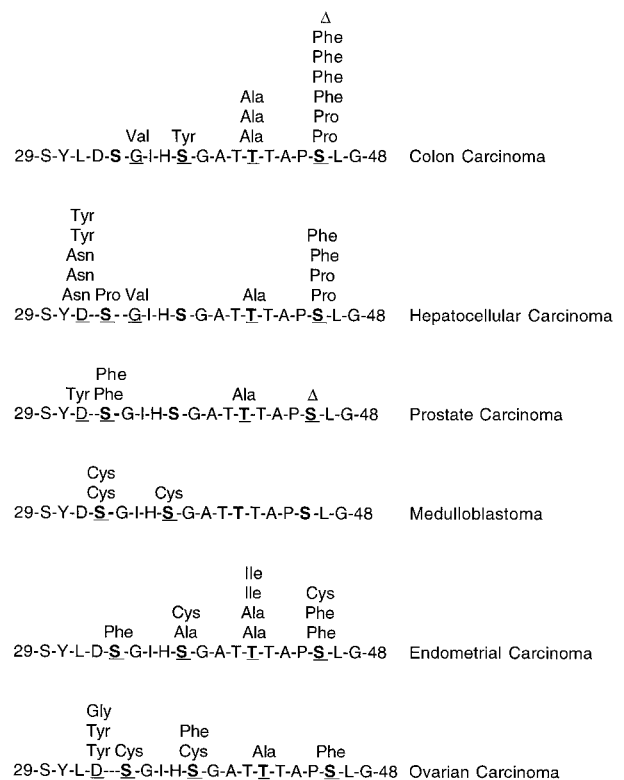
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                                       Δ
                                      Phe
                                      Phe
                                      Phe
                             Ala      Phe
                             Ala      Pro
              Val    Tyr     Ala      Pro
29-S-Y-L-D-S-G-I-H-S-G-A-T-T-T-A-P-S-L-G-48        Colon Carcinoma


              Tyr
              Tyr                      Phe
              Asn                      Phe
              Asn                      Pro
              Asn Pro Val     Ala      Pro
29-S-Y-D--S--G-I-H-S-G-A-T-T-T-A-P-S-L-G-48        Hepatocellular Carcinoma


                 Phe
              Tyr Phe          Ala       Δ
29-S-Y-D--S-G-I-H-S-G-A-T-T-T-A-P-S-L-G-48         Prostate Carcinoma


              Cys
              Cys     Cys
29-S-Y-D-S-G-I-H-S-G-A-T-T-T-A-P-S-L-G-48          Medulloblastoma


                             Ile
                             Ile       Cys
                     Cys     Ala       Phe
              Phe    Ala     Ala       Phe
29-S-Y-L-D-S-G-I-H-S-G-A-T-T-T-A-P-S-L-G-48        Endometrial Carcinoma


              Gly
              Tyr          Phe
              Tyr Cys      Cys     Ala      Phe
29-S-Y-L-D---S-G-I-H-S-G-A-T-T-T-A-P-S-L-G-48 Ovarian Carcinoma
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Figure 2. Reported point mutation in exon 3 of β-catenin gene in human carcinoma samples (excluding preinvasive lesions and cell lines). Bold letters indicate phosphorylated amino acids by GSK-3β. Underlined letters indicate mutated amino acids (D Asp; S Ser; G Gly; T Thr) (refs. 17, 21, 23–27, and this series).

in the sequence for GSK-3β phosphorylation have been described by others.[18,20,22,23,25] The entire coding sequence of β-catenin has been analyzed in colon[22] and hepatocellular carcinomas[25] and cell lines derived from colon cancer[20] and melanoma.[18] These studies indicate that interstitial deletions affecting exon 3 may occur, whereas mutations outside exon 3 of the β-catenin gene are infrequent.[20] Thus insertional deletion affecting exon 3 has been reported in 10% of colon carcinomas without APC mutations,[22] and in 3% of hepatocellular carcinomas.[25]

It is also possible that the nucleocytoplasmic pattern of β-catenin immunoexpression in the two cases without β-catenin gene mutations was due to some alteration in other molecules of the β-catenin-Tcf signaling pathway. At present, it appears that the intracellular pool of β-catenin is regulated by an active GSK-3β that recognizes an APC-β-catenin complex.[8] The role of APC in this complex is still poorly understood, but it has been suggested that APC may be involved in directing β-catenin to proteasomes.[15] APC contains β-catenin binding and degradation sites, as well as binding sites for GSK-3β. Thus the cytoplasmic β-catenin pool can be increased in response not only to mutations in the β-catenin gene, but also to extracellular Wg/Wnt signaling,[39] which would inactivate GSK-3β, or to genetic defects affecting GSK-3β or APC. Expression of different Wnt factors has been described in human carcinomas,[40,41] but to date their effect in β-catenin levels has not been reported. In addition, the expres-

sion pattern for these factors in ovarian cancer remains to be established. The role of GSK-3β in human oncogenesis is not well known. Recent studies do not report any alterations of GSK-3β in medulloblastomas and colon carcinomas, some of which have mutations of the β-catenin gene.[23,27] Increased β-catenin cytoplasmic and nuclear levels can also be observed in cases of APC inactivation (ie, mutations of APC in colorectal polyps and carcinomas[19,42] and other conditions, such as sporadic aggressive fibromatosis[43]). Loss of heterozygosity has been reported at the APC locus in sporadic ovary carcinomas with a frequency of 50% in a series that included only four endometrioid carcinomas[44]; nevertheless, this seems to be a late event in tumor progression and does not correlate with mutation in the APC gene.[44]

We did not observe β-catenin mutations in serous, mucinous clear cell, or mixed carcinomas, including four mixed tumors with an endometrioid component and two serous carcinomas with focal nuclear β-catenin immunoexpression. In these two cases we did not perform microdissection of the nuclear immunostained areas, and we could not exclude mutations in these cases. β-Catenin mutations have been reported as occurring focally in some prostate cancers, suggesting that these mutations may occur during tumor progression.[24] Interestingly, two of the colon cancers reported by Kitaeva et al[21] and two colon cancer cell lines (HCT 116 and SW48), which have β-catenin gene mutations, also show a replication error-positive phenotype, suggesting that the activating mutations in β-catenin that occur in some tumors may be associated with an underlying defect in DNA mismatch repair.

In the present series, the significant prognostic value of the traditional clinicopathological variables (grade and stage) as predictors of relapse-free and overall survival at 5 years in early-stage ovarian cancer[2–4] was confirmed. In addition, the β-catenin expression pattern was selected as an independent prognostic marker, indicating that cases with nuclear expression have an excellent prognosis, with no relapses or deaths among them. Obviously, these results must be taken as preliminary because of the small number of cases, but they do suggest β-catenin as a potential prognostic marker that could be analyzed in larger series from different institutions. Although we did not observe any deaths among endometrioid carcinomas with a nuclear β-catenin expression pattern, three of the four patients with endometrioid carcinomas with only membranous expression died. These findings suggest two oncogenic pathways in endometrioid carcinomas with different clinical implications. Identification of molecular defects in the subgroup of patients without nuclear β-catenin expression and with apparently poor prognosis would be helpful in selecting a specific therapy for each patient.

In addition to the role of β-catenin in signal transduction, this molecule has a pivotal role in cell adhesion through its participation in the cadherin/catenin complex of adherens junctions. Changes in the expression and structure of β-catenin lead to loss of adhesiveness and may promote invasiveness and metastasis in tumor cells.[9,12,13] Mutations of β-catenin disrupting the interaction between E-cadherin and α-catenin have been described in tumor cell lines that grow as loose aggregates or single cells.[45,46] In human tumors, the expression of membranous β-catenin has been analyzed in head and neck,[47] esophageal,[48,49] gastric,[50] colon,[51] breast,[52] and ovarian[53,54] carcinomas. It has been observed that 88% of head and neck squamous cell carcinomas, 70% of esophageal carcinomas, 50% of gastric carcinomas, and 30% of infiltrating ductal carcinomas of the breast show reduced membranous β-catenin expression. In some reports, reduced expression of β-catenin has been correlated with poorly differentiated tumors, advanced stage, and poor survival.[48,50]

Davis et al[54] observed reduced immunohistochemical β-catenin expression in 21% of a series of 34 ovarian carcinomas, but Fujimoto et al[53] did not find differences in mRNA β-catenin expression between normal ovary tissue and ovarian carcinomas. We observed reduced membranous β-catenin expression in 78% of our ovarian carcinomas. β-Catenin expression did not correlate with histological type, tumor differentiation, progression (stage), or survival. However, it should be remembered that other molecules of the cadherin/catenin complex may be more important than β-catenin in modulating adhesion in ovarian carcinomas. For example, α-catenin was reduced in approximately 60% of cases in a large series of stage I and II ovarian carcinomas, and severe reduction of α-catenin expression ($<$30%) in stage I ovarian carcinomas was a predictor of poor prognosis in the univariate and multivariate analysis.[55]

In summary, the pattern of β-catenin expression differs between ovarian carcinomas of differing histological types. APC-β-catenin-Tcf signaling pathway activation secondary to oncogenic β-catenin mutation is characteristic of a group of endometrioid carcinomas with nuclear β-catenin expression and a good prognosis, most of which originate in previous benign or borderline lesions. Endometrioid carcinomas with only membrane expression of β-catenin seem to represent a different subgroup of carcinomas that is not associated with the β-catenin signaling pathway and which, probably, has a worse prognosis. Determination of the β-catenin expression pattern in early-stage ovarian cancer might prove to be a useful marker for selecting low-risk patients.

Acknowledgments

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