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β-catenin expression, DNA ploidy and clinicopathological features in ovarian cancer: A study in 253 patients

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Abstract

The *CTNNB1* gene and its product β-catenin, a regulator of the Wnt signalling pathway, is often mutated and deregulated in human malignancies. Down stream targets of the Wnt signalling pathway are linked to genomic instability. In this study, the impact of β-catenin expression on genomic instability in ovarian carcinoma, as determined by DNA ploidy, was investigated. Expression of β-catenin was examined by immunohistochemistry in 253 ovarian carcinomas. The results were related to genomic instability and clinicopathological features of the patients. Membrane associated staining of β-catenin was detected in nearly all cases with no correlation to clinical parameters. Most of the samples also had cytoplasmic (84%), while only 13% had nuclear β-catenin localisation. A significant association between β-catenin expression (cytoplasmic and nuclear) and histological subtype and degree of differentiation was observed. Nuclear β-catenin was almost exclusively present in endometroid carcinomas. 53% of all endometroid tumours were positive for nuclear β-catenin expression ($P < 0.0001$). Mucinous carcinomas had the highest degree of cytoplasmic β-catenin expression (92%), followed by endometroid (92%), mixed (90%), serous (82%), unclassified adenocarcinomas (81%), carcinomas clear cell and (70%), ($P = 0.01$). Tumours with differentiation grade 1 (16%) and 2 (24%) had higher nuclear β-catenin expression than grade 3 and clear cell carcinomas (6%) ($P = 0.012$). Better prognostic outcome was found for patients with nuclear β-catenin localisation as compared to the cases without ($P = 0.027$). In conclusion, the study showed no correlation between β-catenin expression in ovarian carcinoma and FIGO stage and genomic instability as determined by DNA ploidy status. However, nuclear β-catenin expression was strongly associated with endometroid histological subtype. Finally, in ovarian cancer, although β-catenin staining seems to be of prognostic importance with respect to nuclear staining in univariate analysis, only DNA ploidy status, histological grade and FIGO staging were of independent prognostic significance in multivariate analysis.

Keywords: β-catenin; Ovarian carcinoma; DNA ploidy and genomic instability

1. Introduction

The β-catenin gene (*CTNNB1*) encodes for β-catenin and apart from its well-defined role in cellular adhesion it is also a component of the Wnt signalling pathway [1]. Wnt signalling events result in β-catenin accumulation and transcriptional activation of target genes. Deregulation of β-catenin signalling is an important event in the genesis of malignancies, such as colon cancer, melanomas, ovarian carcinomas and others [2]. The levels of β-catenin are regulated by a protein complex containing adenomatous polyposis coli (APC) tumour suppressor

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protein, glycogen synthase kinase-3β (GSK-3β), and Axin [3,4]. This protein complex promotes degradation of free cytosolic β-catenin via GSK-3β mediated phosphorylation of amino-terminal β-catenin sequences and its degradation by the ubiquitin–proteasome pathway. When the Wnt pathway is activated, degradation of β-catenin is inhibited, allowing β-catenin entry into the nucleus where it interacts with Tcf/Lef transcription regulator proteins [3]. Within the nucleus, β-catenin transcriptionally activates target genes such as *c-myc* [5], *cyclin D* [6,7], *matrix metalloproteinase-7* (*MMP-7*) [8,9], and *cyclooxygenase*-2 [10]. The β-catenin binding domain of APC alone is found to be sufficient to inhibit tumour growth by blocking the nuclear translocation of β-catenin and down regulate target gene expression of the Wnt pathway [11].

Ovarian carcinomas are a heterogenous group of neoplasms with different biological potential and clinical outcome (reviewed by Shih and Kurman [12]). We have recently reported on the prognostic value of genomic instability as determined by DNA ploidy analysis in ovarian carcinomas [13]. It has been reported that mutations in the *APC* tumour suppressor gene have been found to cause chromosomal instability [14,15], and activation of Wnt signalling pathway is involved in the development of polyploidy in solid tumours [16]. Wnt pathway mutations, whether in the *CTNNB1* or *APC* genes, lead to elevated β-catenin levels in the cytoplasm and nucleus [17,18] and thereby the transcription of target genes [19]. The expression of some target proteins of the Wnt pathway such as c-myc [20,21], cyclin D [22] and cyclooxygenase-2 [23], have all been linked to genomic instability. To elucidate the role of β-catenin in genomic instability, we have studied β-catenin expression and correlated the findings with the genomic instability measured by DNA ploidy status in 253 patients with ovarian carcinomas. The results were also correlated to clinical and pathological features of the patients.

2. Patients and methods

2.1. Patients

The clinical characteristics of the patients have been published previously [13]. Briefly, the population consisted of 253 patients diagnosed with ovarian cancer of serous, mucinous, clear cell, endometroid, mixed, small cell, and unclassified type of carcinoma, diagnosed between 1982 and 1989. Clinical follow up data were available for all patients until death or 31 December 1998.

2.2. Immunohistochemistry

Formalin fixed, paraffin embedded sections (4 μm), were mounted onto silane-coated slides. After air-drying at 37 °C for 24 h, slides were deparaffinised and rehydrated. The slides were pre-treated for 2×5 min in microwave oven (EDTA, pH 8), before incubation with primary antibody against β-catenin (1:3000, Transduction Laboratories) over night. Staining was performed with peroxidase labelled avidin–biotin. Negative controls were treated in the same way, with the exclusion of primary antibodies. Positive controls consisted of carcinomas known to be immunoreactive for the studied marker.

2.3. Interpretation of immunostaining

A Nikon Eclipse 800 microscope (Nikon Corporation, Tokyo, Japan) with a magnification of 200× was used to evaluate the sections. The presence of β-catenin immunostaining in carcinoma cells was evaluated by two of the authors (B.R and J.M.N.) without knowledge of the clinical outcome. Membranous, cytoplasmic and nuclear β-catenin localisation were evaluated in each case. For the extent of staining the following scale was used: 0 no staining, 1 = 1–5%, 2 = 6–25%, 3 = 26–50% and 4 = >50% positive tumour cells.

2.4. DNA ploidy analysis

DNA ploidy analysis was performed by image cytometry with Fairfield DNA ploidy system (Fairfield Imaging, Ltd., Nottingham, UK) as previously described in detail [13,24].

2.5. Statistical analysis

Comparison of groups was performed by Pearson χ^2 tests. Survival of patients was estimated by Kaplan–Meyer analysis and the covariates were analysed by the log rank test for univariate analysis. A Cox proportional hazards regression model was used for multivariate evaluation. *P*-values <0.05 were considered statistically significant. The correlation of the scoring of β-catenin between the two authors was calculated by κ-statistics. SPSS (version 10.1, SPSS, Chicago) statistical software was used for the calculations.

3. Results

3.1. Expression of β-catenin

The localisation and intensity of membranous, cytoplasmic and nuclear β-catenin staining in 253 cases of ovarian carcinomas is summarised in Table 1. Most of the tumours were positive for membranous (98%) and cytoplasmic (84%) β-catenin expression, while only 13% of the tumours had nuclear β-catenin expression. Most of the tumours (83%) showed high intensity (>50%)

Table 1
β-catenin expression in 253 patients with ovarian carcinomas

Staining intensity (%)	Membranous staining *n* (%)	Cytoplasmic staining *n* (%)	Nuclear staining *n* (%)
0	6 (2.4)	41 (16.2)	220 (87.0)
1–5	7 (2.8)	17 (6.7)	5 (2.0)
6–25	18 (7.1)	42 (16.6)	6 (2.4)
26–50	12 (4.7)	17 (6.7)	4 (1.6)
>50	210 (83.0)	136 (53.8)	18 (7.1)

n, number of samples stained from total.

membranous β-catenin expression; the corresponding numbers for cytoplasmic and nuclear staining were 54% and 7%, respectively. Fig. 1 shows typical membranous, cytoplasmic and nuclear β-catenin expression.

3.2. Correlation between β-catenin expression and FIGO staging, histological subtype, degree of differentiation and DNA ploidy classification

The association between β-catenin expression and the variables FIGO staging, histological subtype, degree of differentiation and DNA ploidy classification are presented in Table 2. No significant association between membranous β-catenin expression and the other variables was found. There was significant association between cytoplasmic and nuclear β-catenin expression and histological subtypes and degree of differentiation. Nuclear β-catenin was almost exclusively observed in endometroid carcinomas, where 53% of the tumours stained positive ($P < 0.0001$). Mucinous carcinomas (93%) had the highest degree of cytoplasmic β-catenin positive samples followed by endometroid (92%) mixed (90%), serous (82%), unclassified adenocarcinomas (81%) and clear cell carcinomas (70%) ($P = 0.01$). Significantly more tumours with differentiation grade 1 (16%) and 2 (24%) had positive nuclear β-catenin expression than grade 3 and clear cell carcinomas (6%) ($P = 0.012$). There was no correlation between β-catenin expression and genomic instability as determined by DNA ploidy status or FIGO stage.

3.3. Survival analysis

In ovarian carcinomas, nuclear β-catenin staining seemed to be of prognostic importance, while cytoplasmic or membranous staining were not (Fig. 2). There was a significant difference with respect to recurrence

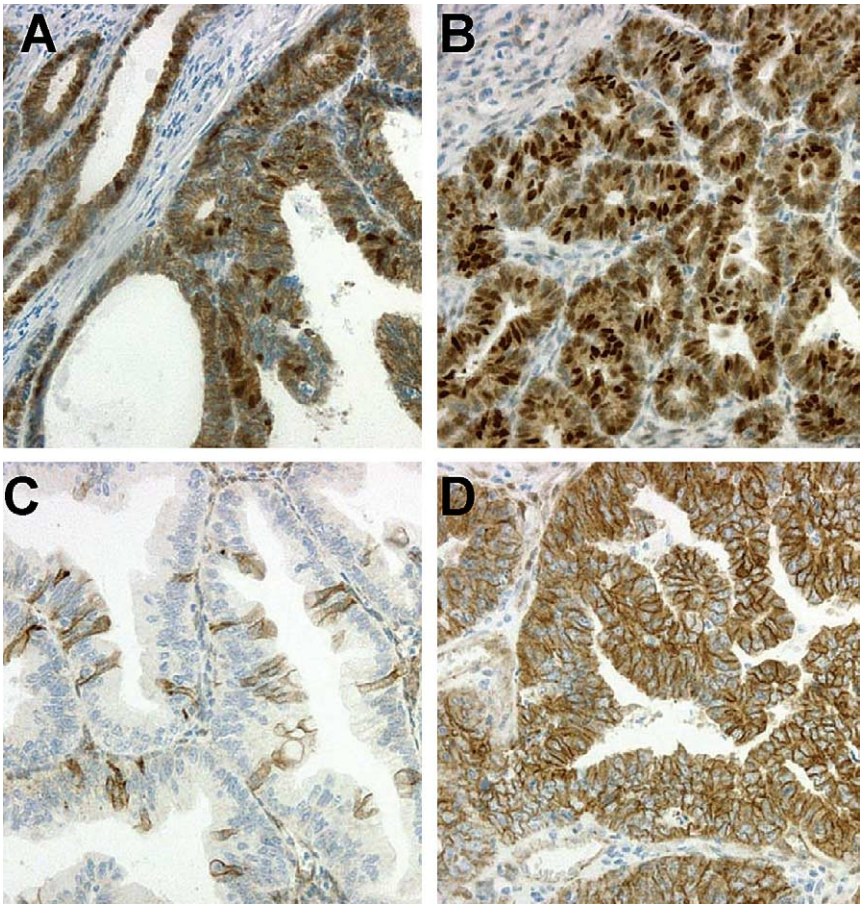

Fig. 1. Representative immunohistochemical analysis of β-catenin expression. Tumour with (A) membranous, cytoplasmic and nuclear (B) cytoplasmic and nuclear (C) membranous and (D) membranous and cytoplasmic β-catenin expression.

Table 2
β-catenin expression, FIGO staging, histologic typing and grading, and DNA ploidy in 253 patients with ovarian carcinoma[a]

Characteristic		*N*	Membranous β-catenin staining			Cytoplasmic β-catenin staining			Nuclear β-catenin staining		
			−ve *n* (%)	+ve *n* (%)	*P*-value[b]	−ve *n* (%)	+ve *n* (%)	*P*-value	−ve *n* (%)	+ve *n* (%)	*P*-value
FIGO	Ia	86	3 (3)	83 (97)	NS	14 (16)	72 (84)	NS	75 (87)	11 (13)	NS
	Ib, Ic	167	3 (2)	164 (98)		27 (16)	140 (84)		145 (87)	22 (13)	
Histological subtype	Serous	60	0 (0)	60 (100)	NS	11 (18)	49 (82)	0.01	59 (98)	1 (2)	<0.000
	Mucinous	57	3 (5)	54 (95)		4 (7)	53 (93)		55 (96)	2 (4)	
	Clear cell	57	0 (0)	57 (100)		17 (30)	40 (70)		55 (96)	2 (4)	
	Endometroid	51	14 3 (6)	48 (94)		4 (8)	47 (92)		24 (47)	27 (53)	
	Unclassified adenocarcinoma	16	0 (0)	16 (100)		3 (19)	13 (81)		15 (94)	1 (6)	
	Mixed[c]	10	0 (0)	10 (100)		1 (10)	9 (90)		10 (100)	0 (0)	
	Small cell	2	0 (0)	2 (100)		1 (50)	1 (50)		2 (100)	0 (0)	
Differentiation	Grade 1	106	3 (3)	103 (97)	NS	15 (7)	91 (93)	0.01	89 (84)	17 (16)	0.012
	Grade 2	38	0 (0)	38 (100)		1 (3)	37 (97)		29 (76)	9 (24)	
	Grade3/clear cell	109	3 (3)	106 (97)		25 (23)	84 (77)		102 (94)	7 (6)	
DNA ploidy classification	Diploid/tetraploid	162	3 (2)	159 (98)	NS	21 (13)	141 (87)	NS	138 (85)	24 (15)	NS
	Aneuploid/polyploid	91	3 (3)	88 (97)		20 (22)	71 (78)		82 (90)	9 (10)	

N, number of total samples in group.
n, number of samples from *N*.
NS, not significant.
[a] Because of rounding, percentages may not total 100.
[b] Pearson χ^2.
[c] Mixed without a clear cell component.

free survival in cases with nuclear β-catenin staining in comparison to negative nuclear staining (Fig. 2, $P = 0.027$ by the log rank test). Overall, cancer specific survival of patients with positive and negative nuclear β-catenin tumours was 88% and 68%, respectively. Cytoplasmic and membranous β-catenin staining did not seem to have any prognostic impact (Fig. 2, $P = 0.230$ and 0.997, respectively). In multivariate analysis, DNA ploidy classification, histologic grade and FIGO stage were of independent prognostic significance for recurrence free survival (Table 3). Patients without nuclear β-catenin expression had 1.8 higher risk for recurrence of disease than patients with it, but the finding did not reach statistical significance (Table 3).

3.4. Interobserver variability assessed by computing κ-values

Correlations of positive *versus* negative expression had high reproducibility for nuclear ($\kappa = 0.861$) and

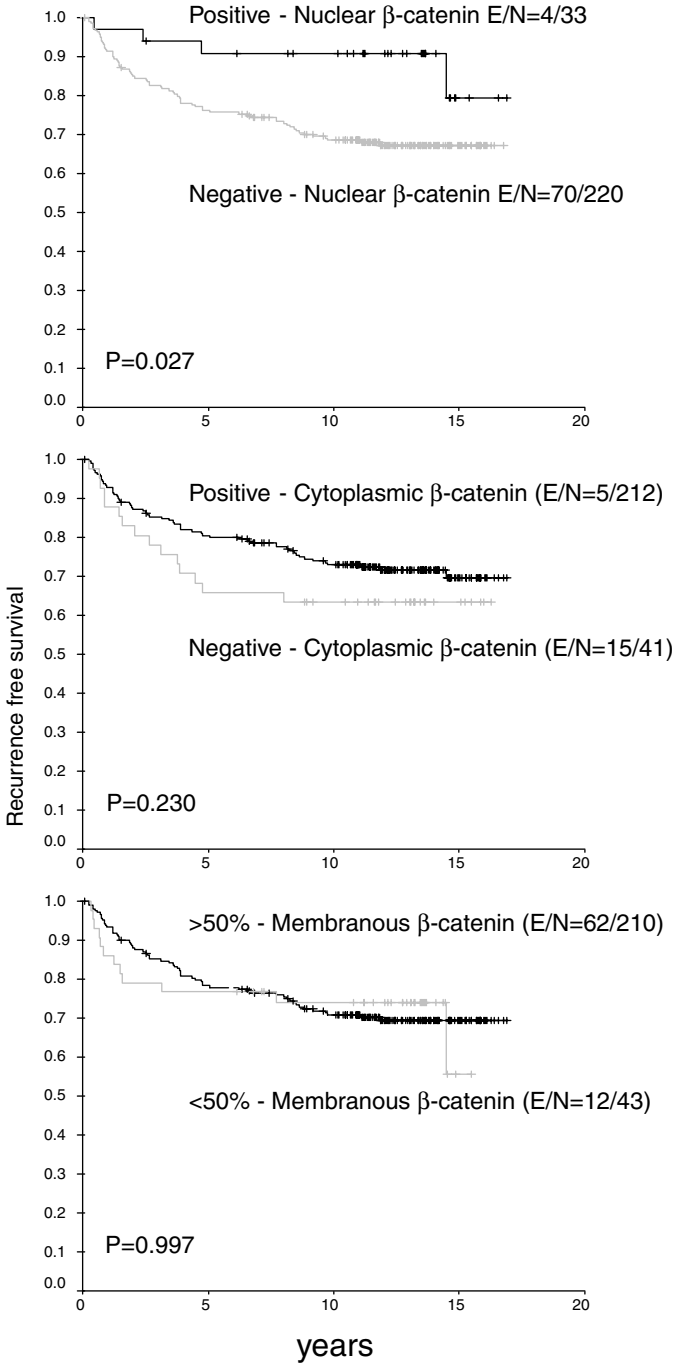

Fig. 2. Recurrence free survival related to β-catenin expression in 253 patients with ovarian carcinoma. Nuclear, cytoplasmic, and membranous β-catenin expression is shown in top, middle and bottom, respectively. E = event, N = total number.

Table 3
Variables for survival in multivariate analysis

Characteristics	Recurrence free survival/hazard ratio (95% CI)	*P*
DNA ploidy classification		
Diploid/tetraploid	1	<0.000
Polyploid/aneuploid	7.9 (4.4–14.2)	
Degree of differentiation		
Grade 1/grade 2	1	0.004
Grade 3/clear cell	2.2 (1.3–3.8)	
FIGO stage		
Ia	1.0	0.009
Ib-c	2.2 (1.2–3.9)	
Nuclear β-catenin		
Positive	1	0.253
Negative	1.8 (0.7–5.2)	

FIGO, International Federation of Obstetrics and Gynecology; CI, confidence interval.

average for membranous ($\kappa = 0.388$) and low for cytoplasmic ($\kappa = 0.090$) β-catenin expression, respectively. Corresponding numbers for scoring with 5 categories were: nuclear (0.588), membranous (0.411) and cytoplasmic (0.269) β-catenin expression.

The inter-observer variation for scoring nuclear and membranous expression of β-catenin was acceptable and indicated specific staining. However, the same was not true for cytoplasmic staining where the inter-observer variation was high, indicating unspecific staining.

4. Discussion

Mutations of the *CTNNB1* gene or other components in the Wnt signalling pathway, leading to over expression and nuclear translocation of β-catenin, are important molecular events in the carcinogenesis of several types of solid tumours [18,25]. In this combined investigation of nuclear β-catenin expression, FIGO stage, and genomic instability as determined by DNA ploidy status in ovarian cancer, our novel findings show a lack of correlation between β-catenin expression and either genomic instability or FIGO stage. Further, nuclear β-catenin staining, but not cytoplasmic or membranous, was associated with improved survival, and occurred preferentially in endometroid carcinoma. The results suggest that one mechanism for tumourigenesis in endometroid ovarian carcinomas may be by modulation of the Wnt signalling pathway.

Our results are in agreement with previous studies demonstrating nuclear β-catenin expression in 16–86% ovary endometroid carcinomas [18,26–31]. Variation in patient numbers and use of different antibodies may explain the spread in β-catenin tumour expression. Among other histological subtypes nuclear β-catenin expression was very low (6/202, 3%) and corroborates results from other groups [25,32,33]. An exception to this is finding of nuclear β-catenin expression in 12% of 105 serious carcinomas examined [34]. The low nuclear β-catenin expression in grade 3/clear cell carcinomas found in this study are in agreement with the data of Wright *et al.* [30] showing more *CTNNB1* mutations in high-grade tumours. Mutation in the *CTNNB1* gene is found to result in β-catenin translocation to the nucleus, but nuclear β-catenin expression has also been observed without evidence of *CTNNB1* mutation [28]. Since almost all of the nuclear positive tumours in our study are of endometroid histological subtype, differentiation grade is probably not an independent prognostic factor for this subset of ovarian carcinoma.

Here, we demonstrate that the 33 patients with nuclear β-catenin localisation had higher cancer specific survival during long term follow up as compared to patients without nuclear β-catenin expression in univariate analyses. When performing Kaplan–Meyer analysis on only the endometroid carcinomas, the finding is no longer significant, probably due to lower sample size. Even though the relative hazard for developing a recurrence of disease was almost 2 times higher for patients without nuclear β-catenin expression in multivariate analysis, this finding did not reach statistical significance due to variation and small group size. Only DNA ploidy analysis, histological grade and FIGO stage were of independent prognostic significance for relapse free survival in multivariate analyses. An association between accumulated β-catenin nuclear expression and favourable prognosis has been reported in ovarian [35] and hepatocellular carcinoma [36]. Elevated levels of mutated β-catenin in the nucleus were similar to our findings, and shown to be a significant predictor of prolonged survival. As nuclear entry of β-catenin is required for *Tcf* transcription [11], staining may be used as evidence of β-catenin over expression, resulting in increased cellular proliferation. One may speculate that increasing proliferation activity renders the cells more vulnerable to systemic treatment in endometroid ovarian tumours, which remains to be investigated. A recent study by Faleiro-Rodrigues and colleagues [37] found that loss of β-catenin was associated with poor survival in ovarian carcinomas. They did not, in contrast to us, examine nuclear β-catenin expression and included few endometroid carcinomas. In contrast to our findings Wong and colleagues [25] report that nuclear β-catenin localisation was associated with metastases in colorectal cancer patients. Lin and colleagues [38] reported a significant correlation between β-catenin nuclear localisation and a decrease in survival in breast carcinoma patients. Together these findings underline the complexity of effects in Wnt signalling, and show that the effect of β-catenin might be tissue specific and diverse in different tissues.

Previously the relationship between microsatellite instability (MI) and mutations in the β-catenin gene had been examined [28,39]. No significant association between MI and β-catenin was found. Down stream target proteins of the Wnt signalling pathway such as c-myc [21], cyclin D [22] and cyclooxygenase-2 [23] have been linked to genomic instability. In our study we found no relationship between genomic instability as measured by DNA ploidy analysis and β-catenin expression. The established link between genomic instability and c-myc, cyclin D and cyclooxygenase-2 appears not to be due to deregulation of β-catenin. In addition, our results gave no support to the theory that lost membranous β-catenin expression is concomitant with disease progression.

In conclusion, we found no relationship between β-catenin expression in ovarian carcinoma and FIGO stage and genomic instability as measured by DNA ploidy analysis. However, nuclear β-catenin expression was strongly associated with the endometroid histological subtype. Finally, in ovarian carcinoma, although β-catenin staining seems to be of prognostic importance with respect to nuclear staining in univariate analyses, only DNA ploidy analyses, histological grade and FIGO staging were of independent prognostic significance in multivariate analyses.

Conflict of interest statement

None declared.

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