ORIGINAL ARTICLE

Prognostic significance of E-cadherin–catenin complex in epithelial ovarian cancer

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Objective: To clarify the prognostic role of E-cadherin and β - and γ -catenins, and their relation to CD44 in epithelial ovarian carcinoma.

Methods: The expression of E-cadherin and β - and γ -catenins was analysed immunohistochemically in 305 primary epithelial ovarian cancers and 44 metastases, and related to CD44 expression, clinicopathological factors, and the patients' survival.

Results: Reduced cell surface expression of E-cadherin, β -catenin, and γ -catenin was particularly frequent in serous and endometrioid histological types. Reduced cell surface expression of E-cadherin and β -catenin was also associated with poor differentiation. Nuclear positivity of β -catenin was associated with high CD44 expression, endometrioid histology, and local stage of the tumour, whereas nuclear γ -catenin expression was associated with serous histology and poor differentiation. In the univariate analysis, preserved cell surface β -catenin expression in the whole study material and nuclear expression of β - and γ -catenins in the subgroup of endometrioid ovarian cancers were predictors of better 10 year disease related survival. Preserved cell surface expression of E-cadherin and β -catenin predicted favourable recurrence-free survival. These statistical significances were not retained in multivariate analysis.

Conclusions: The correlation between nuclear β -catenin and CD44 indicates that β -catenin may regulate the transcription of CD44 in epithelial ovarian cancer. E-cadherin–catenin complex members are associated with the prognosis of patients with epithelial ovarian cancer, but these univariate associations were not strong enough to compete for significance with the traditional clinicopathological factors.

Cell–cell adhesion determines tissue morphogenesis and regulates major cellular processes including motility, growth, differentiation, and survival. The E-cadherin–catenin complex is necessary for the stabilisation of the adhesions and normal physiology of epithelial cells. Adhesion of neighbouring cells is mediated by cadherin, the cytoplasmic domain of which is connected to β - and γ -catenins, which in turn bind to the actin cytoskeleton through α -catenin. Disturbed adhesion often occurs in malignant tumours and may enhance the migration and proliferation of tumour cells, leading to invasion and metastasis.¹

Components of the E-cadherin–catenin complex and their effects on survival have been studied in several malignancies. Abnormal expression of E-cadherin has been shown to be associated with poor survival,^{2–6} and reduced expression of α -, β -, and γ -catenins has been found to predict unfavourable prognosis in various malignancies.^{7–14}

We have previously reported that reduced expression of α -catenin predicts poor prognosis in FIGO stage I ovarian epithelial cancer.¹⁵ Prognostic significance of other components of the E-cadherin–catenin complex has also been suggested in ovarian cancer. Negative E-cadherin^{16–17} and β -catenin,¹⁸ as well as positive γ -catenin expression,¹⁹ have been shown to indicate poor prognosis, while nuclear β -catenin positivity has been implicated in a better prognosis in ovarian cancer.²⁰

Despite these findings, the significance of the E-cadherin–catenin complex in tumour development remains controversial and unconfirmed in larger studies of tumour material.^{4 6 16 19–22} In addition, it has been suggested that E-cadherin and β -catenin interrelate with CD44 expression and functions.^{23–26} This prompted us to clarify the relation between cell adhesion molecules E-cadherin, with its β - and

γ -catenin companions, and CD44, as well as the prognostic significance of the E-cadherin–catenin complex in a large group of epithelial ovarian cancer patients.

METHODS

Patients

We analysed 305 specimens from women treated and diagnosed for epithelial ovarian malignancy at Kuopio University Hospital and Jyväskylä Central Hospital, Finland, between 1976 and 1992, with a follow up until January 2004. Patients who were treated before operation, or who were not operated on, were not included. Patients who died because of any postoperative complications (deaths within one month after the operation) were not included in the survival analyses. Forty four of the patients also had metastatic lesions.

Staging of the tumours was based on the International Federation of Gynecologists and Obstetricians (FIGO) standards. In addition to operative treatment, seven patients (2%) received postoperative radiotherapy, 223 (73%) received postoperative chemotherapy, and 38 (13%) received both of these adjuvant treatments. Up to January 2004, disease recurrence had been observed in 75 patients (25%) and no recurrence in 91 (30%); the tumour remained or was progressing in 139 patients (45%). The median follow up time for all patients ($n = 305$) was 28 months (range 1 to 334), and for patients still alive ($n = 78$, 26%), 139 months (12 to 334). At the end of the follow up, 195 patients (64%) had died because of the ovarian carcinoma, while 32 (10%)

Abbreviations: ABC, avidin-biotin-peroxidase complex; FIGO, International Federation of Gynecologists and Obstetricians

Table 1 Clinicopathological characteristics of the patients (n = 305)

Characteristic	
Age (years), median	62
Age range (years)	18 to 85
Histological grade	
1	44 (14%)
2	104 (34%)
3	157 (52%)
Histological type	
Serous	107 (35%)
Mucinous	33 (11%)
Endometrioid	80 (26%)
Clear cell	32 (11%)
Miscellaneous*	53 (17%)
FIGO stage	
I	84 (28%)
II	47 (15%)
III	142 (47%)
IV	32 (10%)
Primary residual tumour	
No data	26 (8%)
None	122 (40%)
≤ 2 cm	51 (17%)
> 2 cm	106 (35%)
Chemotherapy response	
No data	7 (2%)
PR	38 (13%)
CR	140 (46%)
SD	22 (7%)
PD	54 (18%)
No chemotherapy	44 (14%)
End state	
Dead	227 (74%)
Alive	78 (26%)

Values are n (%) unless stated otherwise.

*Includes 20 mixed epithelial, one Brenner, and 32 unclassified epithelial tumours.

CR, complete response; PD, progressing disease; PR, partial response; SD, stable disease.

had died from other causes. The clinicopathological characteristics of the patients are summarised in table 1.

Histology

Histological typing and grading were re-evaluated according to the WHO classification, as described previously.²⁷

Immunohistochemistry

Five micrometre thick paraffin embedded tissue sections of all tumours were stained immunohistochemically. Deparaffinised and rehydrated sections were heated in a microwave oven for 2×5 minutes (E-cadherin), 6×5 minutes (β-catenin) or 3×5 minutes (γ-catenin) in a citrate buffer, pH 8.0 (E-cadherin) or pH 6.0 (β-catenin and γ-catenin), then incubated in the citrate buffer for 18 minutes, and washed with phosphate buffered saline (PBS) for 2×5 minutes. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide for five minutes, and then the sections were washed with water for 2×5 minutes and with PBS for 2×5 minutes. Non-specific binding was blocked with 1.5% normal horse serum in PBS for 45 minutes (E-cadherin) or for 25 minutes (β-catenin and γ-catenin) at room temperature. The sections were incubated overnight at 4°C with the primary antibody for E-cadherin (mouse monoclonal anti-human E-cadherin, clone HECD-1; Zymed Laboratories, California, USA; 1:100 dilution), for β-catenin (mouse monoclonal anti-human β-catenin, clone 14; Transduction Laboratories, Lexington, Kentucky, USA; 1:1000 dilution), and for γ-catenin (mouse monoclonal anti-human γ-catenin, clone 15; Transduction Laboratories; 1:100 dilution). The negative control was incubated with 1% bovine serum albumin (BSA) in PBS instead of the primary antibody.

Figure 1 (A) A clear cell ovarian cancer showing strong continuous expression of β-catenin on tumour cell membranes; scale bar = 30 μm. (B) A mucinous ovarian cancer with non-continuous membranous staining of β-catenin; scale bar = 30 μm. (C) An endometrioid ovarian cancer showing nuclear β-catenin positivity; scale bar = 50 μm.

Next, the slides were washed with PBS for 2×5 minutes and incubated with the biotinylated secondary antibody (anti-mouse IgG; ABC Vectastain Elite kit, Vector Laboratories, Burlingame, California, USA) for 45 minutes (E-cadherin) or 35 minutes (β-catenin and γ-catenin) at room temperature. After this, slides were washed with PBS for 2×5 minutes, incubated for 50 minutes (E-cadherin) or for 45 minutes (β-catenin and γ-catenin) in preformed avidin-biotinylated peroxidase complex (ABC Vectastain Elite kit, Vector

Laboratories) and washed twice for five minutes with PBS. The colour was developed with diaminobenzidine tetrahydrochloride (DAB) (Sigma, St Louis, Missouri, USA). The slides were counterstained with Mayer's haematoxylin, washed, dehydrated, cleared, and mounted with DePex (BDH, Poole, UK). An external positive control showing strong staining was an ovarian cancer sample for E-cadherin, a thyroid cancer sample for β -catenin, and a lung cancer sample for γ -catenin. The negative control sample (primary antibody omitted) in each staining batch did not show any signal.

CD44 staining

The staining procedure for CD44 was carried out following the above mentioned protocol using the primary antibody (mouse monoclonal anti-human CD44, clone 2C5, R&D Systems, Abingdon, UK, 1:1200 dilution), which recognises all forms of CD44.²⁸ The staining procedure of CD44 has been described in detail previously.²⁹

Evaluation of the stainings (E-cadherin and β - and γ -catenins)

All specimens were analysed by three observers (KAV, SMS, KMR) who were unaware of the clinical outcome. All stainings were evaluated by the same method. The intensity of the staining of cancer cells was categorised into two groups: weak or strong. Strong intensity was equal to the intensity of cancer cell membrane seen in the external positive controls. Weak staining corresponded to the intensities between strong and negative. The staining pattern was considered continuous when membranes around cancer cells showed uninterrupted signal. Non-continuous staining included fragmentary membranous staining and also cytoplasmic staining of cancer cells.

From the total tumour cell area, the percentage area of tumour cells showing strong continuous staining was analysed and further categorised into two groups according to the median percentage of the expression (5% for E-cadherin, β - and γ -catenins): reduced expression, $\leq 5\%$ of the tumour cells expressed strong continuous membranous staining; and preserved expression, $>5\%$ of the tumour cells expressed strong continuous membranous staining. This method of evaluation was modified from that described by Davidson *et al.*¹⁹

The presence of nuclear staining was also analysed and directly graded into two categories: positive or negative. Nuclear staining was considered positive if more than 5% of the tumour cell nuclei were positive. In addition, the presence of strong cytoplasmic expression of E-cadherin and β - and γ -catenins was analysed and graded either present or absent.

Evaluation of CD44

The expression of CD44 was scored as a fraction of positive cancer cells in the whole tumour area and further categorised into two groups according to the median percentage of the expression: low, $\leq 10\%$ of the tumour cells expressed CD44, and high, $>10\%$ of the tumour cells expressed CD44. The

evaluation of CD44 has been described in more detail previously.²⁹

Statistical analyses

The SPSS for Windows 11.0 program (SPSS Inc, Chicago, Illinois, USA) was used for statistical analyses. The relations between continuous variables were evaluated using Wilcoxon tests and Spearman correlation coefficients. Frequency tables were analysed using a χ^2 test. The Kaplan–Meier method was used to evaluate the significant univariate predictors. The differences between curves were analysed using the log-rank test. Multivariate survival analysis was calculated using Cox's proportional hazards model in a forward stepwise manner with the log-likelihood ratio significance test. Disease related survival was defined as the time interval between the date of surgery and the date of death from ovarian cancer. Recurrence-free survival was defined by the time interval between the date of surgery and the date of recurrence. Probability values less than 0.05 were regarded as significant.

Ethics

The research was approved by the research ethics committee of Kuopio University and Kuopio University Hospital.

RESULTS

Membranous expression of E-cadherin and β - and γ -catenins

In all stainings, expression of catenins was located mainly on the tumour cell membrane, either continuously (fig 1A) or non-continuously (fig 1B). The mean percentages of strong continuous membranous expression in primary tumours were 9%, 7%, and 7%, and in metastases, 10%, 5%, and 6% for E-cadherin, β -catenin, and γ -catenin, respectively. In the majority of samples, there was strong continuous membranous staining in 5% or fewer cancer cells (table 2).

Nuclear and cytoplasmic expression of E-cadherin and β - and γ -catenins

Nuclear staining was seen in only a few primary tumour samples ($n = 23$ (8%) for β -catenin (fig 1C), and $n = 52$ (18%) for γ -catenin). Nuclear β - and γ -catenin expressions were not correlated with each other in the primary tumours. In metastases, tumour cell nuclei were negative for E-cadherin, whereas nuclear positivity for β -catenin was observed in one endometrioid tumour sample (2%) and for γ -catenin in two samples (5%). Strong cytoplasmic expression of E-cadherin and β - and γ -catenin was present in 24 (9%), 57 (19%), and 54 (18%) primary tumours, respectively, while five metastases (12%) showed strong cytoplasmic positivity for both E-cadherin and β -catenin, and 12 (28%) for γ -catenin.

Relation between members of the E-cadherin–catenin complex

Strong continuous membrane staining was present for E-cadherin, β -catenin, and γ -catenin, and there were significant correlations between them ($r = 0.39$ between E-cadherin and β -catenin; $r = 0.34$ between E-cadherin and γ -catenin; $r = 0.35$ between β -catenin and γ -catenin; $p < 0.0005$ for all). There were no significant differences in E-cadherin, β -catenin, and γ -catenin expression patterns between primary tumours and metastases.

E-cadherin–catenin complex correlation with CD44

There was no correlation between membranous, nuclear, or cytoplasmic expression patterns of E-cadherin or γ -catenin and the level of CD44 expression in cancer cells. Instead, nuclear β -catenin positivity was associated with high CD44 expression in the primary tumours ($\chi^2 = 8.0$; $p = 0.004$),

Table 2 Reduced ($\leq 5\%$) and preserved ($>5\%$) membranous expressions of E-cadherin, β -catenin, and γ -catenin in primary tumours

Antigen	$\leq 5\%$	$>5\%$	Total (n)
E-Cadherin	204 (72%)	78 (28%)	282
β -Catenin	228 (77%)	68 (23%)	296
γ -Catenin	218 (74%)	75 (26%)	293

Values are n (%).

Table 3 Reduced ($\leq 5\%$) and preserved ($>5\%$) membranous expression of E-cadherin, and clinicopathological correlations

Variable	$\leq 5\%$	$>5\%$	χ^2	df	p Value
Histological grade			7.8	1	0.005
1	23 (62%)	14 (38%)			
2	65 (65%)	35 (35%)			
3	116 (80%)	29 (20%)			
Histological type			29.2	4	<0.0005
Serous	80 (78%)	22 (22%)			
Mucinous	10 (33%)	20 (67%)			
Endometrioid	57 (77%)	17 (23%)			
Clear cell	19 (63%)	11 (37%)			
Miscellaneous	38 (83%)	8 (17%)			
FIGO stage			2.5	1	0.12
I	50 (65%)	27 (35%)			
II	34 (77%)	10 (23%)			
III	98 (73%)	36 (27%)			
IV	22 (81%)	5 (19%)			
Primary residual tumour			1.5	1	0.22
None	78 (69%)	35 (31%)			
≤ 2 cm	32 (70%)	14 (30%)			
>2 cm	76 (77%)	23 (23%)			
Recurrence			6.0	1	0.014
No	51 (62%)	31 (38%)			
Yes	57 (80%)	14 (20%)			
End state			2.7	1	0.10
Dead	158 (75%)	53 (25%)			
Alive	46 (65%)	25 (35%)			

Values are n (%).

df, degrees of freedom; FIGO, International Federation of Gynecologists and Obstetricians.

especially in the endometrioid histological subtype ($\chi^2 = 12.1$; $p < 0.0005$).

E-cadherin-catenin complex and clinicopathological factors

Reduced cell surface expression of E-cadherin and β -catenin was associated with poor differentiation ($\chi^2 = 7.8$; $p = 0.005$, and $\chi^2 = 6.7$; $p = 0.009$, respectively), serous and endometrioid histological types ($\chi^2 = 29.2$ for E-cadherin and $\chi^2 = 56.6$ for β -catenin; $p < 0.0005$ for both), and cancer recurrence ($\chi^2 = 6.0$; $p = 0.014$, $\chi^2 = 3.9$; $p = 0.049$, respectively). In addition,

reduced β -catenin expression on tumour cell membrane was associated with advanced FIGO stage ($\chi^2 = 9.6$; $p = 0.002$) and large (>2 cm) primary residual tumour ($\chi^2 = 7.0$; $p = 0.008$) (tables 3–5).

Nuclear positivity of β -catenin was associated with local stage (FIGO I) of the tumour ($\chi^2 = 9.0$; $p = 0.029$) and endometrioid histological type ($\chi^2 = 26.2$; $p < 0.0005$). Sixteen of 23 primary samples (70%) expressing nuclear β -catenin were of endometrioid type, as well as the one metastatic sample showing nuclear β -catenin positivity. The association between nuclear positivity of β -catenin and local disease

Table 4 Reduced ($\leq 5\%$) and preserved ($>5\%$) membranous expressions of β -catenin, and clinicopathological correlations

Variable	$\leq 5\%$	$>5\%$	χ^2	df	p Value
Histological grade			6.7	1	0.009
1	24 (60%)	16 (40%)			
2	78 (77%)	23 (23%)			
3	126 (81%)	29 (19%)			
Histological type			56.6	4	<0.0005
Serous	90 (85%)	16 (15%)			
Mucinous	10 (34%)	19 (66%)			
Endometrioid	69 (88%)	9 (12%)			
Clear cell	14 (47%)	16 (53%)			
Miscellaneous	45 (85%)	8 (15%)			
FIGO stage			9.6	1	0.002
I	52 (66%)	27 (34%)			
II	32 (71%)	13 (29%)			
III	119 (84%)	23 (16%)			
IV	25 (83%)	5 (17%)			
Primary residual tumour			7.0	1	0.008
None	78 (68%)	37 (32%)			
≤ 2 cm	44 (86%)	7 (14%)			
>2 cm	86 (83%)	18 (17%)			
Recurrence			3.9	1	0.049
No	59 (69%)	27 (31%)			
Yes	60 (82%)	13 (18%)			
End state			2.8	1	0.09
Dead	177 (79%)	46 (21%)			
Alive	51 (70%)	22 (30%)			

Values are n (%).

df, degrees of freedom; FIGO, International Federation of Gynecologists and Obstetricians.

Table 5 Reduced ($\leq 5\%$) and preserved ($>5\%$) membranous expressions of γ -catenin, and clinicopathological correlations

Variable	$\leq 5\%$	$>5\%$	χ^2	df	p Value
Histological grade			1.2	1	0.26
1	27 (69%)	12 (31%)			
2	74 (73%)	28 (27%)			
3	117 (77%)	35 (23%)			
Histological type			22.1	4	<0.0005
Serous	84 (80%)	21 (20%)			
Mucinous	16 (55%)	13 (45%)			
Endometrioid	64 (81%)	15 (19%)			
Clear cell	14 (47%)	16 (53%)			
Miscellaneous	40 (80%)	10 (20%)			
FIGO stage			2.1	1	0.14
I	54 (67%)	27 (33%)			
II	35 (80%)	9 (20%)			
III	106 (77%)	32 (23%)			
IV	23 (77%)	7 (23%)			
Primary residual tumour			6.8	1	0.009
None	80 (70%)	35 (30%)			
≤ 2 cm	32 (67%)	16 (33%)			
>2 cm	90 (85%)	16 (15%)			
Recurrence			3.4	1	0.07
No	56 (68%)	27 (32%)			
Yes	58 (81%)	14 (19%)			
End state			0.9	1	0.35
Dead	166 (76%)	53 (24%)			
Alive	52 (70%)	22 (30%)			

Values are n (%).

df, degrees of freedom; FIGO, International Federation of Gynecologists and Obstetricians.

stage (FIGO I) was also present within the subgroup of 78 endometrioid ovarian cancers ($\chi^2 = 17.6$; $p < 0.0005$).

Reduced membranous expression of γ -catenin was correlated with serous and endometrioid histological types ($\chi^2 = 22.1$; $p < 0.0005$) and large (>2 cm) primary residual tumour ($\chi^2 = 6.8$; $p = 0.009$) (tables 3–5).

Nuclear γ -catenin expression was associated with serous histological type ($\chi^2 = 16.6$; $p = 0.002$) and poor tumour differentiation ($\chi^2 = 9.4$; $p = 0.009$).

Survival

In univariate analysis, preserved β -catenin expression on cell surface indicated a better 10 year disease related survival ($p = 0.035$) (fig 2A). Similarly, preserved membranous expressions of E-cadherin and β -catenin predicted favourable recurrence-free survival ($p = 0.038$ and $p = 0.033$, respectively) (fig 2, panels B and C). Preserved cell surface expression of γ -catenin reached marginal significance in

predicting better recurrence-free survival in univariate analysis ($p = 0.053$), whereas significance was not achieved for disease related survival ($p = 0.46$) (table 6).

Nuclear β - and γ -catenin positivities ($n = 14$ and $n = 10$, respectively) predicted significantly better 10 year disease related survival in univariate analysis in the subgroups of 76 (β -catenin) and 77 (γ -catenin) endometrioid ovarian cancers ($p = 0.008$ and $p = 0.012$, respectively).

Cell surface expressions of E-cadherin, β -catenin, and γ -catenin, as well as histological type and grade, FIGO stage, primary residual tumour, age at diagnosis, and adjuvant chemotherapy, were entered into a Cox multivariate analysis. In addition, nuclear expressions of β - and γ -catenin as well as the above mentioned traditional clinicopathological factors were entered into a Cox multivariate analysis of the endometrioid ovarian cancer subgroup. None of the E-cadherin-catenin complex components retained their statistical significance in predicting disease related survival or

Figure 2 (A) Kaplan–Meier curves for disease related survival of epithelial ovarian cancer patients according to reduced ($\leq 5\%$) and preserved ($>5\%$) membranous expression of β -catenin (log-rank analysis). Kaplan–Meier recurrence-free survival curves of patients with reduced ($\leq 5\%$) and preserved ($>5\%$) membranous expression of E-cadherin (B) and β -catenin (C) (log-rank analysis).

Table 6 Univariate disease related and recurrence-free survival analyses of the patients

Variable	Surviving at 10 years		Recurrence-free at 10 years	
	n (%)	p Value*	n (%)	p Value*
Age (years)	295	0.004	161	0.15
<50	62 (52%)		38 (66%)	
50–65	127 (35%)		71 (48%)	
>65	106 (29%)		52 (52%)	
Histological grade	295	<0.001	161	0.12
1	44 (52%)		29 (66%)	
2	103 (45%)		61 (57%)	
3	148 (26%)		71 (45%)	
Histological type	295	0.048	161	0.006
Serous	106 (28%)		52 (35%)	
Mucinous	33 (58%)		26 (69%)	
Endometrioid	78 (37%)		38 (63%)	
Clear cell	31 (48%)		22 (64%)	
Miscellaneous	47 (30%)		23 (52%)	
FIGO stage	295	<<0.001	161	<0.001
I	83 (72%)		73 (67%)	
II	47 (43%)		34 (56%)	
III	135 (18%)		49 (33%)	
IV	30 (10%)		5 (40%)	
Primary residual tumour	271	<<0.001	153	<0.001
None	121 (68%)		103 (66%)	
≤ 2 cm	51 (20%)		29 (34%)	
>2 cm	99 (12%)		21 (24%)	
Adjuvant chemotherapy	292	0.11	160	0.23
Platin based	161 (30%)		79 (48%)	
Non-platin	97 (41%)		58 (57%)	
None	34 (50%)		23 (65%)	
E-Cadherin in cancer cells (strong continuous)	273	0.12	149	0.038
≤ 5%	198 (33%)		106 (46%)	
>5%	75 (43%)		43 (67%)	
β-Catenin in cancer cells (strong continuous)	286	0.035	154	0.033
≤ 5%	219 (31%)		115 (48%)	
>5%	67 (51%)		39 (67%)	
γ-Catenin in cancer cells (strong continuous)	283	0.46	150	0.053
≤ 5%	210 (34%)		110 (47%)	
>5%	73 (40%)		40 (65%)	

*Log-rank analysis.

FIGO, International Federation of Gynecologists and Obstetricians.

recurrence-free survival in the cohort as a whole. In addition, nuclear expressions of β - and γ -catenin did not have an independent prognostic role in the endometrioid ovarian cancers subgroup. The significant factors in multivariate analysis of the whole cohort were the presence of primary residual tumour (risk ratio (RR) = 3.53, 95% confidence interval (CI), 2.051 to 6.089; $p < 0.0005$), differentiation grade (RR = 1.58, 95% CI, 1.128 to 2.221; $p = 0.007$), and FIGO stage of the tumour (RR = 1.89, 95% CI, 1.147 to 3.113; $p = 0.009$) for disease related survival; and the presence of primary residual tumour (RR = 2.92, 95% CI, 1.744 to 4.879; $p < 0.0005$) and histological type of tumour (RR = 0.44, 95% CI, 0.265 to 0.738; $p = 0.002$) for recurrence-free survival. In multivariate analysis of endometrioid ovarian cancers the only significant factor predicting disease related survival was FIGO stage (RR = 6.74, 95% CI, 3.029 to 15.010; $p < 0.0005$).

DISCUSSION

Previous studies on E-cadherin-catenin complex in ovarian carcinoma are quite limited, and have left the prognostic value of this complex unclear. Some of the studies have

suggested that the complex has prognostic significance,^{16–20} while others have not.^{22–30} Thus we wanted to clarify the role of the complex in a large sample of epithelial ovarian cancers. In our study univariate analysis indicated that the reduced cell surface expression of β -catenin and E-cadherin predicted poor disease related or recurrence-free survival, or both. However, in multivariate analysis traditional clinicopathological factors (such as the presence of primary residual tumour, FIGO stage, differentiation grade, and histological type of tumour) remained the most important. In addition, we show for the first time that nuclear β -catenin is associated with the adhesion factor CD44 in epithelial ovarian cancer.

We found that strong continuous membranous staining of E-cadherin and β - and γ -catenins was limited to 5% or fewer of the cancer cells in the majority of cases, in line with previous studies.^{18–19} In the present study, expression of the E-cadherin-catenin complex in primary tumours did not differ from that in their metastases, in contrast to α -catenin which had lower expression in metastases than in the primary tumours in our earlier study.¹⁵ On the other hand, upregulation of E-cadherin and the different catenins has been found

in metastatic lesions, and it has been suggested that this facilitates implantation and establishment of metastatic lesions.^{31–32} These apparently contradictory findings probably reflect the multiple mechanisms involved in the regulation of E-cadherin–catenin complex expression during tumorigenesis and metastasis. For example, it has been proposed that hypoxia induced transcriptional repression of E-cadherin expression,³³ E-cadherin gene promoter methylation³⁴ (for example, in cadherin switching regulation³⁵), and mutations in the β -catenin gene, especially in endometrioid ovarian carcinomas,^{20–36–38} take part in this process.

In the present study, E-cadherin and β - and γ -catenin expressions were intercorrelated, and the expression of γ -catenin was also correlated with that of α -catenin (data not shown),¹⁵ findings that are all in line with earlier data,^{31–32–39} though there was a previous report of a lack of association between E-cadherin and β -catenin.³⁰ E-cadherin–catenin complex expression was associated with the histological subtype of the tumour, and all members of the complex except γ -catenin correlated significantly with the differentiation of the tumour as well. The correlation with histological subtype and differentiation has been controversial in previous studies, some suggesting an association^{19–30–39} and others not.^{16–32–40} The large amount of material available in the present study strongly suggested that mucinous tumours were more likely to have preserved expression of the E-cadherin–catenin complex compared with tumours of the serous and endometrioid histological subtypes. This may be a reflection of molecular pathways underlying tumorigenesis in the different histological subtypes.⁴¹

A relation between the E-cadherin–catenin complex and CD44 has been suggested previously.^{23–26–42–43} The present study indicated a significant positive correlation between nuclear expression of β -catenin and high CD44 expression, in line with the observation that the transcription of CD44s and CD44v6 is regulated by β -catenin/Tcf-4.²⁵ The increase in the expression of Wnt/Wingless-signalling pathway components in malignant ovarian tumours⁴⁴ may lead to the accumulation of cytoplasmic β -catenin, increased binding of β -catenin to TCF/LEF transcription factors, and enhanced expression of CD44. Dysregulation of β -catenin, leading to its nuclear accumulation, is a common feature of ovarian carcinomas of endometrioid type.⁴⁵ Indeed, in our series most (16 of 23) ovarian cancers with nuclear β -catenin positivity were of the endometrioid type, and the association between nuclear β -catenin expression and CD44 was seen particularly in the endometrioid ovarian carcinomas.

The importance of the E-cadherin–catenin complex as an indicator of ovarian cancer patients' prognosis has been studied previously, but with fewer patients than in our study. Reduced membranous expression of E-cadherin has predicted shorter survival in univariate¹⁶ and multivariate analysis,¹⁷ in line with our findings, while no difference in survival was associated with this variable in another study.³⁰ Previous results on cell surface β -catenin expression are also contradictory. In some studies, membranous expression of β -catenin was unrelated to survival,^{20–30} while another study showed that β -catenin negativity indicated poor overall survival,¹⁸ in keeping with the present observations using univariate analysis. Nuclear β -catenin positivity in our material predicted better 10 year disease related survival in the subgroup of patients with endometrioid ovarian cancer. A similar finding has been reported in early stage ovarian cancers,²⁰ but an opposite trend emerged in advanced stage, high grade serous ovarian cancers.²² However, in the present study neither membranous nor nuclear β -catenin expression retained statistical significance in multivariate analysis, suggesting that the associations are too weak to resist the confounding factors that come from different evaluation

Take home messages

- Reduced cell surface expression of E-cadherin predicts poor recurrence-free survival from epithelial ovarian cancer
- Reduced β -catenin expression predicts both disease related and recurrence-free survival at 10 years

methods and clinicopathological features of the cancer materials used by different groups.

To the best of our knowledge, nuclear γ -catenin expression has not been reported previously in immunohistochemical ovarian cancer studies. In fact, mutations of the γ -catenin gene are thought to be infrequent in ovarian carcinomas.^{45–46} However, nuclear localisation of γ -catenin has been suggested by *in vitro* studies,^{47–48} and immunohistochemically observed nuclear γ -catenin expression has been described in endometrial carcinoma.⁴⁹ Despite some structural and functional similarities to β -catenin, critical differences between the functions of β - and γ -catenin in Wnt signalling and target gene regulation are obvious, and it has been suggested that γ -catenin functions as an oncogene⁵⁰ or a suppressor of tumorigenicity.⁵¹ In our series, nuclear positivity for γ -catenin showed a statistically non-significant trend towards poor survival in the whole study material (data not shown). Importantly, in the subgroup of endometrioid ovarian cancers, nuclear γ -catenin positivity predicted a better disease related survival at 10 years in univariate analysis. It is apparent that the importance of γ -catenin in ovarian cancer materials remains to be settled in the future.

Conclusions

Nuclear β -catenin positivity correlates with CD44 expression, and reduced E-cadherin–catenin complex expression on the cell surface is associated with serous and endometrioid histological subtype and poor differentiation of the tumour. Reduced cell surface expression of E-cadherin predicts poor recurrence-free survival, and reduced β -catenin expression predicts both disease related and recurrence-free survival at 10 years. In addition, positive nuclear β - and γ -catenin expressions are predictors of favourable disease related survival in the subgroup of endometrioid ovarian carcinomas. However, it appears at present that traditional clinicopathological factors remain more important for evaluating the prognosis of ovarian cancer patients.

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