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Original Article

Claudin-4 Expression is Associated With Survival in Ovarian Cancer But Not With Chemotherapy Response

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Summary: The tight junction protein claudin-4 has been reported to be overexpressed in advanced ovarian cancer. We investigated the prognostic significance of claudin-4 overexpression and whether claudin-4 expression could predict platinum response in primary ovarian carcinoma (OC). Claudin-4 expression was evaluated by immunohistochemistry in a tissue microarray of 140 OCs. Multivariable Cox-regression models were used to assess the effect of claudin-4 overexpression on progression-free survival and overall survival (OS). Kaplan-Meier survival analyses and the logrank test were performed comparing claudin-4 high and low groups. The association between claudin-4 expression and platinum resistance was assessed using risk ratios and the Pearson χ^2 test. A dataset of >1500 epithelial ovarian cancers was used to study the association between *CLDN4* mRNA and survival. Of 140 evaluable cases, 71 (51%) displayed high claudin-4 expression. Claudin-4 overexpression predicted shorter 5-yr progression-free survival and OS in univariable analyses [hazard ratio (HR) = 1.6 (1.1–2.5), $P = 0.020$ and HR = 1.6 (1.0–2.4), $P = 0.041$, respectively]. Hazard of relapse was similar [HR = 1.5 (1.0–2.4)] after adjustment for age, stage, type, and *BRCA1/2* status in a multivariable analysis, but the evidence was slightly weaker ($P = 0.076$). Validation in an external cohort confirmed the association between high expression of *CLDN4* and poor 10-yr OS [HR = 1.3 (1.1–1.5), $P < 0.001$]. However, no confident association between claudin-4 and platinum sensitivity was found in our cohort [risk ratio = 1.2 (0.7–2.0), $P = 0.3$]. These findings suggest that high expression of claudin-4 may have a prognostic value in OC. The role of claudin-4 in the development of platinum resistance remains unclear. **Key Words:** Claudin-4—Ovarian carcinoma—Platinum resistance—Prognostic factor.

Ovarian cancer is one of the leading causes of cancer death among women due to difficulties in both diagnosis and therapy. Because of the insidious onset of the disease and the lack of reliable screening methods, two thirds of patients present with advanced stage disease upon diagnosis (1). The 5-yr

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relative survival rate is 27% for patients with advanced disease (stage III-IV) (2). Despite efforts aimed at early detection and new therapeutic approaches, the poor survival persists and may be explained by the heterogeneity and poorly understood pathogenesis of ovarian carcinoma (OC).

Platinum compounds comprise the most active chemotherapy available and constitute standard treatment after cytoreductive surgery for the majority of women diagnosed with ovarian cancer (3). First-line chemotherapy with platinum-based chemotherapy yields response rates of $>80\%$. However, nearly all patients relapse and eventually develop platinum resistance (4). A biomarker capable of predicting platinum resistance at primary surgery could make it possible to identify patients who may not be suitable for platinum-based therapy, and thus be spared its side effects, or who could be eligible for other treatment options.

Claudins belong to a multigene family, with ~ 24 members, and are considered one of the main tight junction (TJ) forming proteins (5,6). A TJ acts as a cellular barrier and is involved in paracellular permeability and cell polarity (7,8). Since the discovery of claudins in 1998, many studies have aimed to characterize the claudin family, revealing that the specific combination and coexpression of different claudin species determines the TJ barrier characteristics in a tissue-specific manner (9,10). TJ structure and function are often altered in human carcinomas, where TJ loss can contribute to cancer progression (11). For example, claudin-1 has been found to be downregulated in breast cancer, and claudin-2 is downregulated in breast and prostate cancer (12,13). In contrast, claudin-4 has been reported to be upregulated in pancreatic, colorectal, gastric, breast, prostate, and ovarian cancers (13–20). Previous studies have reported increased expression of claudins 3 and 4 in OC compared with normal ovarian surface epithelium and benign ovarian tumors (20–22).

Claudin-4 has been proposed as a possible diagnostic and prognostic biomarker in OC (3,23). However, there are limited and contradictory data regarding the involvement of claudins in chemotherapy resistance (24–26). Therefore, in this study we investigated the role of claudin-4 as a potential prognostic marker in 140 patients with primary OC. The relationship between platinum resistance and claudin-4 expression was also investigated.

MATERIALS AND METHODS

Patients

A total of 128 patients with OC were recruited consecutively in the southern Swedish health care region between 1998 and 2000 (27). In addition, 18 patients with OC were recruited at the oncogenetic counseling at Lund University Hospital (Sweden) between 1981 and 1997. Six patients with unknown primary tumor or missing follow-up information were excluded. Thus, a cohort of 140 OC patients was assessed for claudin-4 protein expression.

The study was approved by the Ethics Committee at Lund University, Sweden, waiving the requirement for informed consent. The histologic subtype and grade were determined according to WHO 2014 (28), and all tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO) criteria (29). Tumors were also classified into types I and II on the basis of both histology and grade (30). Platinum resistance was defined as primary progressive disease or recurrence within 6 mo of completing platinum-based therapy, the definition used in clinical practice today.

The majority of the tumors (70%) were of serous histology, followed by endometrioid, mucinous, and clear cell histologies. Approximately 75% of the patients were diagnosed with advanced stage (III/IV) disease, 64% were classified as type II, and 31/140 (22%) were carriers of a germline *BRCA1* or *BRCA2* mutation. Platinum-based chemotherapy was administered postoperatively to 132 patients: Carboplatin (AUC5) combined with paclitaxel (175 mg/m^2) to 70 patients, Carboplatin (AUC5) combined with cyclophosphamide (500 mg/m^2) to 25 patients, and unspecified platinum-based chemotherapy to 36 patients. Thirty-nine of the 132 patients who received chemotherapy (30%) were platinum resistant. Seven patients with early-stage (IA-B) disease and type I tumors were excluded from the study of claudin-4 expression as a treatment predictive marker for platinum response, as they had not received platinum-based therapy, in accordance with current guidelines. One patient was excluded from the same analysis because she received non–platinum-based chemotherapy postoperatively.

Data from a cohort of 1582 OC patients available through the online tool Kaplan-Meier Plotter (31) were used for validation.

Tissue Microarray (TMA) Construction and Immunohistochemistry

We used a TMA with 0.6 mm triplicate core needle biopsies from viable tumor areas (27). Sections, 3–4 μm in thickness, were deparaffinized, rehydrated, and stained. Antigen retrieval was performed using Dako

Target retrieval solution of pH 6 (Dako A/S, Glostrup, Denmark) in a pressure cooker 2100 Retriever (Histolab Products AB, Gothenburg, Sweden). The sections were incubated with a claudin-4 mouse monoclonal antibody (Cat.No. 32-9400; Invitrogen Corporation, Camarillo) at a 1:100 dilution for 30 min. Immunohistochemical reactions were performed using the Autostainer Plus with the Dako REAL EnVision Detection system (Dako). Normal colon tissue was included as a positive control. The negative controls were ovarian stroma and follicle cells as well as smooth muscle and adipose tissue, all reported as negative for claudin-4 expression in The Human Protein Atlas (32). We also stained whole-tissue sections with benign ovary, fallopian tube, and endometrium, as well as 3 cases of serous cystadenomas.

A semiquantitative analysis of claudin-4 was performed by 2 blinded investigators following recommendations from a senior gynecologic pathologist (A.M.) and on the basis of the similar type of expression pattern observed for HER2. Claudin-4 staining was distinct and predominantly confined to the cell membrane. Two staining patterns were observed: (i) punctate and partial staining around the membrane; and (ii) complete membrane staining, covering the circumference of the membrane surface. Results from the core with the highest/strongest positivity were recorded into 5 subgroups on the basis of staining pattern and fraction of stained cancer cells (0: no staining, 1: $<50\%$ of cells with punctate/partial membrane staining, 2: 50%–75% of cells with punctate/partial membrane staining, 3: 50%–75% of cells with complete membrane staining, 4: $>75\%$ of cells with complete membrane staining).

As claudin-4 was expressed in the majority of OCs examined and no consensus for cutoff was available in the literature, an exploratory cutoff was used in the present study. Tumors with a $\geq 3+$ score were considered to have high expression and the rest had low expression. This cutoff was established on the basis of the percentage of patients with primary platinum-resistant disease and the 2 staining patterns observed, before evaluating the effect on prognosis or platinum resistance.

Statistical Analyses

The prognostic value of claudin-4 was investigated using 5-yr progression-free survival (PFS) time and 5-yr overall survival (OS) time as endpoints. PFS time was defined as the time interval between date of diagnosis and the first sign of disease recurrence (clinical and/or radiologic) or death, whichever occurred first. OS time was defined as the time interval between date of diagnosis and death. Of the patients who died within 5 yr after diagnosis, all but 2 died of ovarian cancer.

Statistical analyses were performed with SPSS for Windows version 22. To analyze the probability of a patient being platinum resistant, we calculated the relative risk (risk ratio) of high versus low claudin-4 expression with 95% confidence interval (95% CI). Associations between claudin-4 expression and platinum resistance and other clinical parameters were assessed using the Pearson χ^2 test or the Fisher exact test, except for the ordinal variable stage, which was compared using the Mann-Whitney *U* test. Survival analyses for PFS and OS were performed using the Kaplan-Meier method, and differences between groups were tested using the logrank test. The effect of claudin-4 expression on survival was expressed using hazard ratios (HR) with 95% CI, estimated using univariable (crude effect) and multivariable (adjusting for clinical factors known to influence ovarian cancer survival) Cox regression. These factors included age at diagnosis (≥ 70 y vs. <70), tumor type (II vs. I), stage (III/IV vs. I/II), and *BRCA1/2* mutation status (wild-type vs. mutation) (30,33–35) and were analyzed as binary factors. The cutoff of 70 yr was chosen on the basis of previous studies reporting an inferior outcome among elderly women (33). The classification into type I and II is made on the basis of both histology and grade, and defines 2 fundamentally different groups regarding tumorigenesis and prognosis (30). A large meta-analysis showed better OS and PFS for *BRCA1* and *BRCA2* mutation carriers compared with noncarriers (35). The prognostic factor residual disease following cytoreductive surgery was not included in the analyses, as this information was not documented. Cox regression was performed for the type II tumors alone as a stability analysis. All *P*-values are 2-sided.

RESULTS

Immunohistochemical Staining of Claudin-4

Seven of the 140 OCs displayed no staining, and the distribution in subgroups 1–4 was as follows: 1: n = 32, 2: n = 30, 3: n = 18, 4: n = 53. Thus, 71 cases (51%) displayed high claudin-4 expression. Figure 1 shows representative examples of high and low claudin-4 expression in OC. The expression of claudin-4 (low vs. high) in the different histologic subtypes, stages, and types, as well as in relation to age, *BRCA1/2* mutation

status, and platinum sensitivity, is shown in Table 1. High expression of claudin-4 correlated with high-grade serous histology compared with low-grade serous histology (χ^2 test, $P = 0.037$) and high age at diagnosis (χ^2 test, $P = 0.049$). A correlation between type II tumors and high claudin-4 expression was also observed, but this did not reach statistical significance (χ^2 test, $P = 0.071$). No correlation was found with *BRCA1/2* status (wild-type vs. mutant, χ^2 test, $P = 0.6$) or stage (Mann-Whitney *U* test, $P = 0.1$) (Table 1).

Two whole-tissue sections from a benign ovary with mesothelial hyperplasia revealed negative staining in the mesothelium, including the hyperplastic areas, whereas cortical serous inclusion cysts were strongly positive (Fig. 1). Epithelial cells in the 3 cases of serous cystadenomas also displayed strong claudin-4 expression. Other cell types in the ovary, including stromal and smooth muscle cells, showed no claudin-4 expression in all evaluated cases, in line with the Human Protein Atlas (32). Epithelium from normal fallopian tube and normal endometrium displayed high claudin-4 expression, also in line with the Human Protein Atlas (5 and 10 cases, respectively) (32).

Claudin-4 Expression and Platinum Sensitivity

Among the 132 patients who had received platinum-based chemotherapy, a patient with high claudin-4 expression had a 1.2 (95% CI = 0.7–2.0, $P = 0.3$) times higher risk (risk ratio) of being platinum resistant compared with a patient with low claudin-4 expression (Table 1). No associations with platinum responsiveness were found in separate analyses of type II tumors (n = 86, $P = 0.6$), type I tumors (n = 42, Fisher Exact test, $P = 0.4$), the serous subtype (n = 97, $P = 0.7$), late-stage (III-IV) disease (n = 96, $P = 0.8$), early-stage (I-II) disease (n = 36, Fisher Exact test, $P = 0.2$), or *BRCA1/2* wild-type status (n = 100, $P = 0.2$).

Claudin-4 Expression and Prognosis of OC

Univariable analyses revealed an association between PFS and claudin-4 expression [HR = 1.7 (1.1–2.6), $P = 0.020$] (Table 2), with patients whose tumors expressed high levels of claudin-4 displaying an inferior outcome (Kaplan-Meier survival analysis, Fig. 2). Hazard of relapse was similar [HR = 1.5 (1.0–2.4)] after adjustment for age, stage, type, and *BRCA1/2* status in a multivariable analysis, but the evidence was slightly weaker ($P = 0.076$) (Table 2). A comparable association was observed for OS in the univariable and multivariable analyses [HR = 1.6 (1.0–2.4), $P = 0.041$ and HR = 1.5 (0.9–2.3), $P = 0.1$] (Table 3). Analyses of type II tumors (n = 86) showed a similar HR in multivariable analysis compared with all OCs [1.6 (0.9–2.8),

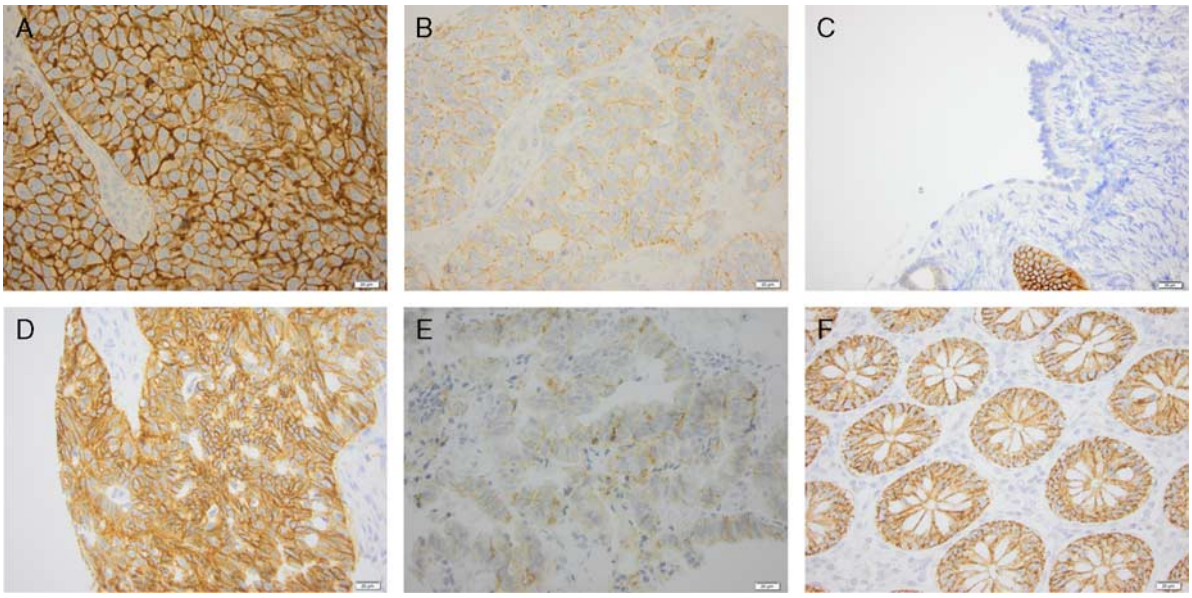

FIG. 1. Immunohistochemical evaluation of claudin-4 in ovarian tissues, including claudin-4 high (A) and low (B) serous ovarian cancer, and claudin-4 high (D) and low (E) endometrioid ovarian cancer. Negative ovarian mesothelium and positive cortical inclusion cyst (C). Positive control, healthy colon (F). Magnification: 40 × .

TABLE 1. *Claudin-4 expression and clinicopathologic parameters*

	N (%)	N (%)		*P**
		Claudin-4 low	Claudin-4 high	
All ECs	140	69 (49)	71 (51)	
Age (yr)				
Mean (range)	59 (26–83)	58 (26–83)	61 (26–83)	
<70	110 (79)	59 (54)	51 (46)	**0.049**
≥70	30 (21)	10 (33)	20 (67)	
BRCA1/2 status				
Mutant	31(22)	14 (45)	17 (55)	0.6
Wild-type	109 (78)	55 (51)	54 (49)	
Histology				
High-grade serous	80 (70)	36 (45)	44 (55)	**0.037**†
Low-grade serous	18 (13)	13 (72)	5 (28)	
Endometrioid FIGO 3	5 (3.6)	1	4	
Endometrioid FIGO 1/2	16 (11.4)	7	9	
Mucinous	10 (7)	4	6	
Clear cell	5 (3.6)	5	0	
Carcinosarcoma	1	0	1	
Mixed and missing	5			
Tumor type				
I	49 (36)	29 (59)	20 (41)	0.071
II	86 (64)	37 (43)	49 (57)	
Missing	5			
Stage				
I	27 (12)	17 (65)	10 (37)	0.1‡
II	17 (13)	9 (53)	8 (47)	
III	77 (60)	35 (45)	42 (55)	
IV	19 (15)	8 (42)	11 (58)	
Platinum responsiveness				
Sensitive	91 (70)	43 (50)	44 (50)	0.3
Resistant	39 (30)	17 (44)	22 (56)	
Missing	2			

Bold values are statistically significant P value <0.05.
*χ^2 test between claudin-4 low and high groups.
†χ^2 test comparing the high-grade and low-grade serous ovarian carcinomas.
‡Mann-Whitney U test between claudin-4 low and high groups.

$P = 0.078$ for PFS and 1.6 (0.9–2.7), $P = 0.086$ for OS].

To extend our findings, we investigated the role of *CLDN4* mRNA levels in a cohort of 1582 OC patients available through the online tool Kaplan-Meier Plotter (31). The online tool comprises gene expression data and survival information from 13 independent public OC data sets (Gene Expression Omnibus and The Cancer Genome Atlas, n = 28-565, 2015 version). The best performing threshold was used as cutoff, where 1105 patients (70%) were classified as *CLDN4* low and 477 (30%) as *CLDN4* high. These results were in line with our findings, as *CLDN4*-high patients displayed worse 5-yr OS in a univariable analysis [HR = 1.3 (1.1–1.5), $P<0.001$] compared with *CLDN4*-low patients. Interestingly, the results were consistent after 10 yr of follow-up [see Fig. Supplemental Digital Content 1, http://links.lww.com/IJGP/A57, HR = 1.3 (1. 2–1.5), $P<0.001$ for 10-yr OS]. However, a similar difference in 10-yr PFS was not observed [HR = 1.2 (1.0–1.3), $P = 0.064$].

DISCUSSION

A previous study has reported low or absent claudin-4 expression in normal ovarian surface epithelium and ovarian cystadenomas, in contrast to the high expression reported in OC, which according to the authors supports the involvement of claudin-4 in malignant transformation (20). We also found absence of expression in ovarian surface mesothelium, and interestingly also in the hyperplastic changes. In contrast to the former study, we found strong positive expression in cortical inclusion cysts (CIC) and serous cystadenomas. Furthermore, we also found strong positive expression in normal fallopian tube epithelium, consistent with data reported in the Human Protein Atlas (32).

TABLE 2. *Univariable and multivariable analyses of progression-free survival*

		5-yr PFS univariable Cox		5-yr PFS multivariable Cox	
	N (events)	HR (95% CI)	*P*	HR (95% CI)	*P*
Claudin-4 expression					
Low	64 (38)	1			
High	70 (55)	1.7 (1.1–2.6)	**0.020**	1.5 (1.0–2.3)	0.076
Age at diagnosis (yr)					
<70	107 (71)	1			
≥70	27 (22)	1.5 (0.9–2.4)	0.1	1.1 (0.6–1.8)	0.7
BRCA1/2 status					
Mutant	29 (18)	1			
Wild-type	105 (75)	1.7 (1.0–3.1)	0.052	2.6 (1.5–4.6)	**0.001**
Stage					
I/II	40 (11)	1			
III/IV	94 (82)	6.2 (3.3–11.7)	<**0.001**	4.4 (2.2–8.8)	<**0.001**
Type					
I	47 (20)	1			
II	82 (68)	2.9 (1.8–4.8)	<**0.001**	2.2 (1.2–3.8)	**0.023**

Bold values are statistically significant *P* value <0.05.
CI indicates confidence interval; HR, hazard ratio; PFS, progression-free survival.

A new paradigm for the pathogenesis of ovarian serous carcinoma, which questions the traditional model of ovarian mesothelium as the cell of origin for serous neoplasia, has been proposed. Cogent arguments support the role of fallopian tube epithelium in the genesis of both low and high-grade serous OCs (36–43). Serous tubal intraepithelial carcinomas found in prophylactic salpingo-oophorectomies in *BRCA* mutation carriers and sporadic cases of pelvic high-grade serous carcinoma are this far the best evidence of this extraovarian hypothesis (40,42,43). Furthermore, a new model of serous tumorigenesis proposes that normal tubal epithelium may shed and implant on the ovarian surface, thereby forming CICs. CICs and serous cystadenomas follow a benign course in most cases, but in some cases they can eventually lead to low and sometimes high-grade serous tumors (42). Thus, no conclusion regarding the involvement of claudin-4 in malignant transformation should be made on the basis of the comparison with ovarian mesothelium (40).

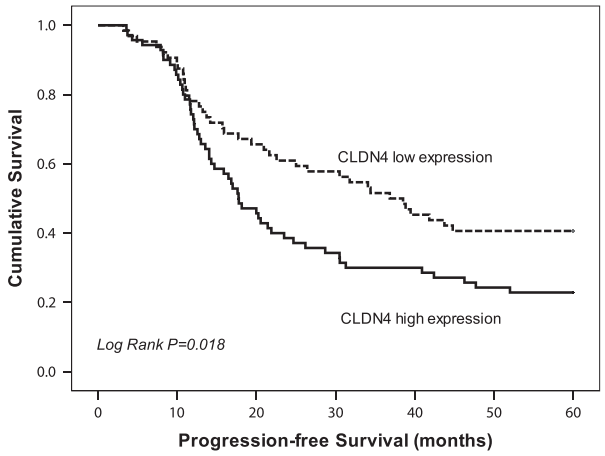

FIG. 2. Kaplan-Meier survival analysis. Association between progression-free survival (PFS) and the level of claudin-4 expression. Patients with high claudin-4 expression (solid line, n = 70) had shorter PFS than those with low expression (dotted line, n = 64). Median PFS 18 versus 37 mo. Logrank *P* = 0.018.

In our study, fallopian tube epithelium, as well as CICs and serous cystadenomas, displayed high claudin-4 expression, arguing against the upregulation of claudin-4 in malignant transformation. Furthermore, we examined endometrial epithelium, as endometrioid and clear cell tumors have been associated with endometriosis and because their gene expression profiles resemble those of endometrial epithelium (40,43). High endometrial claudin-4 expression was observed, also consistent with the Human Protein Atlas (32).

Decreased expression of claudins in cancer is in agreement with the hypothesis that tumorigenesis is accompanied by TJ disruption and loss of cell-cell adhesion, leading to disease dissemination (11). In contrast, overexpression of claudin-4 has been reported in several cancer types, including OC (20–22,44). Upregulation of *CLDN3* and *CLDN4* has been shown to increase cell invasion and motility in OC cell lines, and, conversely, knockdown caused the opposite effects, suggesting that claudin-3 and 4 may indeed promote ovarian tumorigenesis (45). In the present study, we found that high levels of claudin-4 were associated with decreased PFS and OS. The hazards of relapse or death were similar but not significant when

TABLE 3. *Univariable and multivariable analyses of overall survival*

		5-yr OS univariable Cox		5-yr OS multivariable Cox	
	N (events)	HR (95% CI)	*P*	HR (95% CI)	*P*
Claudin-4 expression					
Low	69 (36)	1			
High	71 (48)	1.6 (1.0–2.4)	**0.041**	1.5 (0.9–2.3)	0.1
Age at diagnosis (yr)					
<70	110 (60)	1			
≥70	30 (24)	1.9 (1.2–2.9)	**0.011**	1.7 (1.0–2.9)	**0.037**
BRCA1/2 status					
Mutant	31 (16)	1			
Wild-type	109 (68)	1.4 (0.8–2.4)	0.2	2.1 (1.2–3.7)	**0.013**
Stage					
I/II	44 (13)	1			
III/IV	96 (71)	4.2 (2.3–7.6)	**<0.001**	2.8 (1.5–5.3)	**0.002**
Type					
I	49 (17)	1			
II	86 (64)	3.0 (1.7–5.1)	**<0.001**	2.1 (1.1–3.8)	**0.020**

Bold values are statistically significant *P* value <0.05.
CI indicates confidence interval; HR, hazard ratio.

adjusting for known prognostic factors, probably due to lack of power. The group with low claudin-4 expression had better prognosis and moreover displayed lower claudin-4 expression than the normal tissue levels used for comparison. Thus, there is a possibility that tumors that downregulate claudin-4 expression during malignant transformation become less aggressive tumors with better prognosis. Our findings also suggest that claudin-4 may have prognostic value.

Currently, the development of resistance can only be determined retrospectively, after patients have experienced the burden and toxicity of ineffective therapy (3). Clinical recurrences that occur within 6 mo after cessation of primary platinum-based chemotherapy are considered platinum resistant. This definition of platinum resistance is a strong predictor of response to second-line therapy, with very low response rates for patients defined as platinum resistant (46,47). A biomarker capable of predicting platinum resistance at primary surgery could enable the identification of patients not suitable for platinum-based therapy and who may thus be spared its side effects. Furthermore, patients with overexpression of claudin-4 and platinum-resistant disease may be candidates for clinical trials, including claudin-4-targeted therapy, as claudin-4 is potentially druggable using the C-terminal fragment of *Clostridium perfringens* enterotoxin (24–26). Such a biomarker could therefore provide the basis for individualization of therapy and improved outcome. In the current study we attempted to establish whether claudin-4 might be predictive of platinum sensitivity. Only a few reports are available on the association between platinum resistance and claudin-4 expression, and the results are contradictory. A proteomic study found 7-fold increased levels of claudin-4 protein in a platinum-resistant cell line compared with the sensitive parental line (3). Two studies have reported associations between high levels of claudin-4 protein and platinum resistance (24,26), whereas a third study failed to detect any association with platinum resistance or survival (25). These studies are, however, small (n = 12–43) and may not be representative of the whole spectrum of OC. In fact, one of the studies reporting significantly higher claudin-4 expression in chemoresistant compared with chemosensitive cases had an overrepresentation of clear cell histology, known to be inherently more chemoresistant (10/43 cases, 23%), compared with our cohort (4/130 cases, 3%) (26). The association between platinum resistance and claudin-4 may hence be confounded by the histologic type, emphasizing the importance of stratification of histologic and probably also molecular subtypes when investigating potential treatment predictive biomarkers in OC. The other study compared *CLDN4* mRNA levels at primary surgery from 6 patients with chemotherapy naive serous OC with chemotherapy resistant tumors from 6 patients with recurrent disease, showing *CLDN4* upregulation at recurrence (24). In contrast, we investigated only tumor tissue from primary surgery, as we were interested in investigating the potential predictive value at first-line chemotherapy. However, pairwise comparisons between primary surgery and relapse may be interesting and could potentially inform treatment decisions in recurrent disease, as there may be a group of patients in whose

tumors claudin-4 is upregulated upon development of platinum resistance. This line of research warrants further attention in larger cohorts with matched primary tumors and relapses. Nevertheless, we did not find a confident association between claudin-4 expression and platinum sensitivity. Our findings are, however, consistent with a previous report showing that claudin-4 expression, determined by qRT-PCR and IHC, did not differ between platinum-sensitive and platinum-resistant tumors in a cohort of 36 patients with high-grade, advanced-stage serous ovarian cancer (25). Furthermore, the definition of platinum resistance differed in the previously reported studies. Taken together, we did not find claudin-4 to be a useful predictive marker for platinum sensitivity in the clinical situation, either in an unselected cohort or within any other subgroup.

Of interest, claudin-4 has been suggested as a potential therapeutic target. Suppression of claudin-4 expression in human serous ovarian cancer cell lines by siRNA led to cellular accumulation of cisplatin and a significant increase in cisplatin sensitivity (26), suggesting that upregulation of claudin-4 expression may contribute to platinum resistance by decreasing drug uptake. Additional mechanisms of drug resistance may nevertheless also be present (48), and it remains to be confirmed whether claudin-4 overexpression is involved. We hypothesize that the heterogeneity and complexity of resistance mechanisms may explain why no differences between resistant and sensitive tumors were observed in the present study.

In conclusion, we identified claudin-4 as a potential prognostic biomarker in OC. Although previous studies have suggested a relationship between claudin-4 and platinum resistance, we were not able to validate this in our larger cohort. The druggable nature of claudin-4 nevertheless makes it appealing to pursue further as a target for circumventing platinum resistance. Further investigations aimed at studying the relationship between claudin-4 and platinum resistance are needed, in addition to validation studies to find application into clinical practice.

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