Claudin-1 is linked to presence of implants and micropapillary pattern in serous borderline epithelial tumours of the ovary

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ABSTRACT

Aims Expression of Claudin-1 has been associated with prognosis in several cancers. Here we investigated the expression pattern of Claudin-1 in borderline tumours of the ovary (BOT).

Methods We analysed a cohort of 114 cases of borderline tumour (BOT). Claudin-1 expression was studied by immunohistochemistry using a polyclonal antibody and was compared with clinical and histopathological characteristics.

Results Strong Claudin-1 expression was found in 30 cases (26.3%) independent of histological subtype. Expression was significantly less frequent in International Federation of Gynecology and Obstetrics (FIGO) stage I (p= 0.045), while the presence of microinvasion did not correlate with Claudin-1 expression. In contrast, we detected a highly significant association of Claudin-1 expression with the presence of peritoneal implants (p=0.003) and micropapillary pattern (p=0.047), which are features exclusively seen in serous BOT. Moreover, when we restricted our analysis to the subtype of serous BOT, the association of Claudin-1 expression with peritoneal implants (p<0.001) and micropapillary pattern (p =0.003) remained highly significant.

Conclusions In conclusion, Claudin-1 expression is associated with the presence of peritoneal implants and micropapillary pattern, which have been shown to be associated with poor prognosis. We speculate that overexpression of Claudin-1 might be linked to the mitogen-activated protein kinase pathway activation in BOT and suggest further studies to define its prognostic and potential therapeutic value.

INTRODUCTION

Borderline tumours (BOTs) of the ovary are a fascinating group of ovarian tumours with a specific behaviour. Their atypical cellular structure and metastatic potential contrasts their less aggressive behaviour, when compared with high-grade serous ovarian tumours, a discrepancy that has been noted since their first description in 1929.[1] They were first segregated as a different group in 2003,[2] and lastly revised in 2014 in the new WHO classification.[3] Similar to invasive carcinoma BOTs are distinguished in six histological subtypes according to the epithelial cell type.

Distinction between BOT and frankly invasive tumours usually does not pose a diagnostic problem. However, serous BOTs (SBOTs) are sometimes associated with microinvasion, lymph node involvement and peritoneal implants. The prognostic effect of the presence of these parameters in SBOT is not fully clear.[2 4–6] The former stratification of invasive and non-invasive implants has been changed in the most recent WHO classification, as any invasive foci should now be considered as low-grade serous carcinoma (LGSC). Otherwise the new classification considers the terms 'serous borderline ovarian tumour' and 'atypical proliferative serous tumour' (APST) as synonymous, and 'SBOT with micropapillary pattern' as synonymous with 'non-invasive low grade carcinoma' (niLGSC).[3] Approximately 30%–47% of patients with SBOT develop extraovarian pelvic and/or intra-abdominal disease in the form of tumour implants.[7] The presence of (non-invasive) implants does not have a prognostic impact, whereas invasive peritoneal disease as associated with LGSC displayed reduced overall survival.[6 8–10] Although BOTs have a more favourable outcome than invasive ovarian cancer, with an overall recurrence rate between 3% and 10%,[11–14] BOT can recur even after several years.[7] The role of BOT in the development of invasive epithelial cancer of the ovary is still unclear. Nevertheless, molecular data seem to confirm the view that invasive LGSC develops in a stepwise fashion from benign cystadenofibroma to classic invasive LGSC via transformations to SBOT (or APST) and serous proliferations with micropapillary pattern.[15 16] At least 7% of SBOT will transform to low-grade carcinoma, which occasionally occurs decades after the primary diagnosis.[7 17 18]

Claudins are a family of integral membrane tight junction proteins that are involved in signal transduction and cellular transport functions.[19] Loss of tight junction integrity seems to play a role in tumourigenesis of solid tumours.[20] Depending on the type of neoplasia, claudin proteins may be reduced, elevated or mislocated in tumour cells compared with normal adjacent cells.[21] To date more than 20 claudins have been cloned and characterised.[22] Claudins may play a cancer-promoting or tumour-suppressing role.[21] Their expression is associated with prognosis in several cancers, suggesting their utility as prognostic factors as well as therapeutic targets.[23 24] Claudin-1 is one of the most dysregulated claudins in human cancers and seems to have a crucial role in various cancers.[25] Here we analysed the expression pattern of Claudin-1 in BOT and its association with clinicopathological

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Table 1 Sample characteristics

Characteristics according to second pathology	n	%
Median age		
46.5 years		
Subtype		
Serous	72	63.2
Mucinous	36	31.6
Endometrioid	2	1.8
Mixed	4	3.5
FIGO stage		
I	86	75.4
II	5	4.3
III	5	4.3
IV	1	1
NA	17	14.9
Micropapillary pattern		
No	87	76.3
Partially	21	18.4
Yes	6	5.3
Microinvasion		
No	113	99.1
Yes	1	0.9
Implants*		
No	104	91.2
Yes	10	8.8

*All non-invasive.
FIGO, International Federation of Gynecology and Obstetrics;NA, not available.

parameters. The main finding was a significant association between strong expression of Claudin-1 and important histological features of BOT.

MATERIALS AND METHODS

Patients and samples

All analyses were performed according to the 'REporting recommendations for tumor MARKer prognostic studies' (REMARK).[26] Samples were processed anonymously. We have previously described a cohort of 137 ovarian BOT samples from the University of Frankfurt with validated diagnosis by two experienced gynaecological pathologists (KE, RA).[27 28] At least one block per centimetre diameter of tumour was examined. Diagnosis and tumour grading were performed according to the current criteria of the WHO.[3] Immunohistochemistry for Claudin-1 was successfully performed for 114 of the samples. The histopathological characteristics of this cohort are shown in table 1.

Histopathological evaluation and immunohistochemistry

Paraffin sections (2 µm) were mounted on Superfrost Plus slides, dewaxed in xylene and rehydrated through graduated ethanol to water. Antigens were retrieved by microwaving sections in 10 mM citrate buffer (pH 6.0) for 20 min at 800 W. Blocking was performed using antibody dilution buffer (DCS-Diagnostics, Hamburg, Germany) at room temperature for 15 min. Subsequently, the Claudin-1 antibody was diluted 1:100 in this buffer. Sections were incubated with the diluted Claudin-1 antibody (Thermo Fisher Scientific, Germany, cat. #RB-9209, lot 9209P1306A) for 1 hour at room temperature. For negative control, the primary antibody was replaced with phosphate-buffered saline. For secondary antibody incubation and detection, the Dako REAL Detection System Alkaline Phosphatase/RED (Dako, Glostrup, Denmark) was used following the protocol of the supplier, and sections were counterstained with Hematoxylin Solution, Gill No 3 (Sigma-Aldrich GHS332). Secondary goat antirabbit antibody (Fast-Red, code: K5005) was purchased from Dianova (Hamburg, Germany).

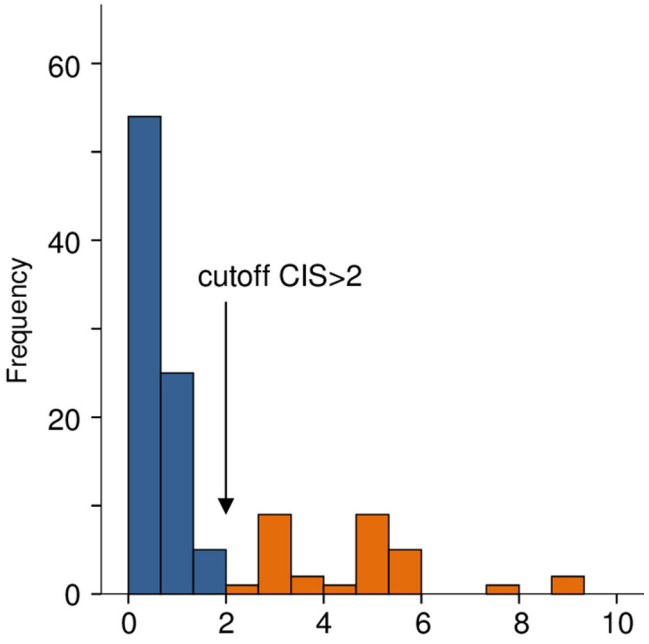

Figure 1 Stratification of borderline tumours into two categories according to the combined immunoreactive score (CIS) of Claudin-1 expression. Based on the observed distribution of CIS, two categories (CIS 0–2 and CIS >2) were chosen for stratification.

Staining intensity (I) was semiquantitatively scored as 'no staining' (0), 'weak' (1), 'medium' (2) or 'strong' (3). A combined immunoreactive score (CIS) was then calculated as the product of staining intensity (I) and the percentage of stained cells (P) with CIS=I×P/30 (thereby normalising the CIS value to the absolute range of 0–10). All assessments were made blinded with respect to clinical patient data. The distribution of the CIS in the cohort is shown in figure 1. Based on this distribution we adopted a cut-off of CIS >2 for our analysis.

Statistical analysis

χ^2 and Fisher's exact tests were used to determine significance of categorical variables and Mann-Whitney U test for analysis of continuous variables. All p values are two-sided and 0.05 was applied as the significance level. Subjects with missing values were excluded from the analyses. All analyses were performed using SPSS Statistics V.22.

RESULTS

Sample characteristics of the cohort

The median age of patients was 46.5 years (34.3–62.8). As shown, most of the samples were either of serous (63.2%, SBOT) or mucinous subtype (31.6%). Regarding the International Federation of Gynecology and Obstetrics (FIGO) stage, most of the patients were classified as stage I (75.4%). Micropapillary pattern and implants were observed solely in SBOT (in 36.1% and 13.9%, respectively, of the patients).

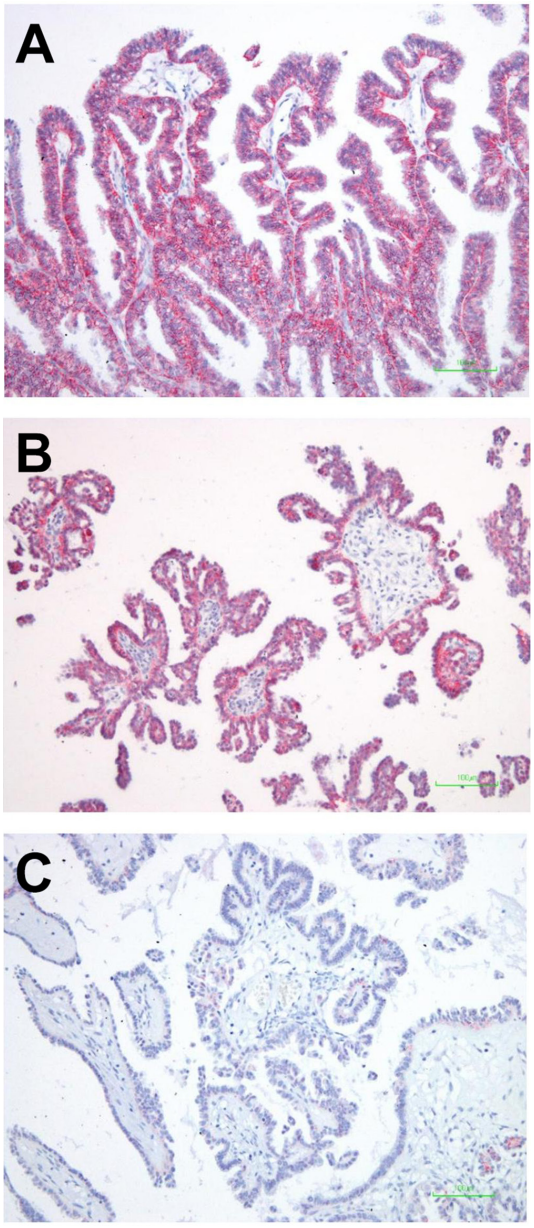

Figure 2 Immunohistochemical detection of Claudin-1 expression in borderline tumours (BOT) of the ovary. Examples of immunohistochemical staining of formalin-fixed, paraffin-embedded borderline tumour tissues using Claudin-1 antibody are shown: mucinous (A) and serous BOT (B) with intense membrane staining. An example of serous BOT negative for Claudin-1 expression is shown in (C). Scale bar 100 µm.

Claudin-1 expression in BOT of the ovary

We next studied Claudin-1 expression by immunohistochemical analysis of tissue samples from all 114 BOTs. Representative examples of Claudin-1 staining results are shown in figure 2. Claudin-1 localised to cell membrane in all tumours with positive staining results. Fibroblasts and endothelial cells stained negative for Claudin-1. Intensity of staining and percentage of stained cells were scored separately and combined as an immunohistochemical score (CIS, see above). Based on the distribution of CIS shown in figure 1, we selected CIS >2 as a cut-off for Claudin-1 expression. This cut-off resulted in 30 cases (26.3%) with positive Claudin-1 expression and 84 cases (73.7%) with no expression. We then compared the expression of Claudin-1 with sample characteristics, as presented in table 2. There was no significant difference in median age between patients with tumours positive or negative for Claudin-1 (p=0.14) (table 2). Furthermore, no significant correlation was found between histological subtype and the expression of Claudin-1. In contrast, Claudin-1 expression was significantly less frequent in FIGO stage I (p=0.045) as 75.6% of these patients did not show Claudin-1 expression. Presence of microinvasion did not correlate with Claudin-1 expression. However, we detected a highly significant association of Claudin-1 expression and the presence of implants (p=0.003), with 70% of cases with implants showing Claudin-1 expression. Furthermore, micropapillary pattern significantly correlated to Claudin-1 expression (p=0.047). Because micropapillary pattern is only observed in SBOT, we separately analysed this subtype. As shown in table 3, both implants (p<0.001) and

Table 2 Claudin-1 expression in borderline tumours of the ovary

Sample characteristics	Claudin-1-negative (CIS <2)	%	Claudin-1-positive (CIS >2)	%	P values
Frequency	84	73.7	30	26.3	0.14
Median age	49.0		39.5		
FIGO stage					
I	65	75.6	21	24.4	**0.045**
II	1	20	4	80	
III	3	60	2	40	
IV	1	100	0		
Subtype					
Serous	54	75	18	25	0.88
Mucinous	26	72.2	10	27.8	
Endometrioid	1	50	1	50	
Mixed	3	75	1	25	
Microinvasion					
No	83	73.5	30	26.5	1.0
Yes	1	100	0		
Implants					
No	81	77.9	23	22.1	**0.003**
Yes	3	30	7	70	
Micropapillary pattern					
No	69	79.3	18	20.7	**0.047**
Partially	12	57.1	9	42.9	
Yes	3	50	3	50	

Significant p-values are given in bold.
CIS, combined immunoreactive score;FIGO, International Federation of Gynecology and Obstetrics.

Table 3 Micropapillary pattern and implants according to Claudin-1 expression in 72 serous borderline tumours of the ovary

	Claudin-1-negative (CIS <2)	%	Claudin-1-positive (CIS >2)	%	P values
Implants					
No	51	82.3	11	17.7	**<0.001**
Yes	3	30	7	70	
Micropapillary pattern					
No	39	84.8	7	15.2	**0.003**
Partially	12	60	8	40	
Yes	3	50	3	50	

Significant p-values are given in bold.
CIS, combined immunoreactive score.

micropapillary pattern (p=0.003) remained significantly associated with Claudin-1 expression in the subgroup of 72 SBOTs.

In addition, to verify the robustness of the obtained results, we also repeated our analyses with two alternative cut-offs for the CIS: slightly lower (CIS >1) and higher (CIS >4), respectively. The association of Claudin-1 expression with the presence of implants in SBOT remained clearly significant with both alternative cut-off values (p=0.005 and p=0.040 for CIS >1 and CIS >4, respectively). The correlation of Claudin-1 expression and micropapillary pattern in BOT showed a strong trend when applying the lower cut-off value (CIS >1, p=0.075) and was clearly significant with the higher cut-off (CIS >4, p=0.008).

Thus, we found that independent of the specific cut-off values applied, Claudin-1 expression is associated with the presence of implants and micropapillary pattern in SBOT.

Discussion

Cancer invasion and metastasis is characterised by changes in the expression of junction proteins and disruption of the cell-to-cell junction. Claudin expression in various tumours alters tight junction structures and function, causing a disruption in cell polarity and decrease in cell adhesion, thus enhancing the invasive and metastatic potential of tumour cells.[29] The exact role of Claudin-1 in tumourigenesis in ovarian cancer is unclear.[30] However, its overexpression has been found to be associated with low degree of differentiation and high rate of invasion in ovarian cancer cell lines.[30]

In the current study, we aimed to investigate the expression pattern of Claudin-1 in BOTs of the ovary and its association with clinicopathological parameters. To our knowledge, this represents the largest analysis of Claudin-1 in BOT to date. Approximately one-third of all examined BOTs (26.3%) expressed Claudin-1, with similar frequency in serous (18 cases) or mucinous (10 cases) histological subtypes. Our results revealed a significant association between strong expression of Claudin-1 and important histological features. The main finding of our study is the correlation of Claudin-1 expression with the presence of peritoneal implants (p<0.001) and micropapillary pattern (p=0.003) in SBOT.

To evaluate the prognostic role of implants, a recently published population-based study from Vang *et al*[31] examined the long-term follow-up of 942 patients with SBOT. This study showed that the presence of implants regardless of being invasive or non-invasive and irrespective of the type of ovarian tumour (APST or niLGSC) was associated with statistically significantly higher rates of subsequent development of serous carcinoma. Furthermore, implants were associated with statistically significantly increased risk of death.[31] On the other hand, presence of a micropapillary architecture in the primary diagnosis of SBOT is a strong predictor of invasive implants.[6] Regarding patients with tumours confined to the ovary harbouring micropapillary growth pattern (niLGSC), the same investigators[31] found a significantly higher rate of subsequent serous carcinoma when compared with typical SBOT. In contrast, du Bois *et al*[32] did not find in their large published cohort study this prognostic effect on recurrence. In another recently published study from McKenney *et al*[33], extraovarian implants with micropapillary architecture were often associated with tissue invasion, but this did not add significant prognostic value when compared with destructive tissue invasion alone.

According to the dualistic model of ovarian carcinogenesis,[34] LGSCs develop gradually from BOT and are characterised by sequence mutations in the KRAS, BRAF and ERBB2 oncogenes, which result in constitutive activation of the mitogen-activated protein kinase (MAPK) signal transduction pathway (MAPK).[35 36] Expression of active MAPK was also detected in some LGSCs as well as high grade serous carcinoma (HGSCs) with wild-type KRAS and BRAF, indicating that the activation of MAPK could be independent from these mutations.[37] Interestingly, Claudin-1 has been linked to the MAPK signal pathway in mice, as treating male mice with MEK 1/2 inhibitor led to a reduction of its expression.[38] These reported findings allow us to speculate that the overexpression of Claudin-1 in the present study might be, at least in part, linked to the activation of the Ras-Raf pathway in BOT. We identified no previous studies linking Claudin-1 expression to the MAP kinase signal pathway in humans.

Our results could contribute to new knowledge in the unclear pathomechanism in the development and behaviour of BOT and low-grade ovarian carcinoma. The strengths of our study include its large sample size, the use of a central pathology as well as the blinded re-evaluation by a second pathologist. Limitations, however, include the retrospective design of the analysis and most importantly the missing follow-up of the patients. Further studies of Claudin-1 expression in BOTs are needed in order to better understand its role as a promising molecular marker in BOT and to define its prognostic and potential therapeutic value.

Take home messages

- Risk factors for invasive recurrence of borderline tumours (BOT) of the ovary are needed.
- Strong Claudin-1 expression was found in 26.3% of BOT linked to features to elevated risk of recurrence as micropapillary pattern and the presence of implants in SBOT.
- Claudin-1 could be a valuable marker to differentiate BOT according to prognosis.

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Contributors KE and RA performed histopathology, diagnosis and data assembly. UH and AE-B conceived the study. UH performed immunohistochemical analyses of the samples and coordinated the study conduct. KE evaluated and scored stained tissue slides and helped in finalising the manuscript. TK carried out the statistical analyses. AE-B, IS, NS and SB provided clinical samples and participated in study conduct. AE-B drafted the manuscript. AE-B, IS and KG finalised the manuscript. All authors approved the final manuscript.

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REFERENCES

1 Taylor HC. Malignant and semimalignant tumors of the ovary. *Surg Gynecol Obstet* 1929;48:204–30.
2 Seidman JD, Soslow RA, Vang R, *et al*. Borderline ovarian tumors: diverse contemporary viewpoints on terminology and diagnostic criteria with illustrative images. *Hum Pathol* 2004;35:918–33.
3 Kurman RJ, Carcangiu ML, Herrington CS. *WHO classification of tumours of female reproductive organs*. 4th edn. Lyon: International Agency for Research on Cancer, 2014.

4 McKenney JK, Balzer BL, Longacre TA. Patterns of stromal invasion in ovarian serous tumors of low malignant potential (borderline tumors): a reevaluation of the concept of stromal microinvasion. *Am J Surg Pathol* 2006;30:1209–21.
5 Silva EG, Deavers MT, Malpica A. Patterns of low-grade serous carcinoma with emphasis on the nonepithelial-lined spaces pattern of invasion and the disorganized orphan papillae. *Int J Gynecol Pathol* 2010;29:507–12.
6 Seidman JD, Kurman RJ. Ovarian serous borderline tumors: a critical review of the literature with emphasis on prognostic indicators. *Hum Pathol* 2000;31:539–57.
7 Longacre TA, McKenney JK, Tazelaar HD, *et al*. Ovarian serous tumors of low malignant potential (borderline tumors): outcome-based study of 276 patients with long-term (> or =5-year) follow-up. *Am J Surg Pathol* 2005;29:707–23.
8 Morice P, Camatte S, Rey A, *et al*. Prognostic factors for patients with advanced stage serous borderline tumours of the ovary. *Ann Oncol* 2003;14:592–8.
9 Bell DA, Longacre TA, Prat J, *et al*. Serous borderline (low malignant potential, atypical proliferative) ovarian tumors: workshop perspectives. *Hum Pathol* 2004;35:934–48.
10 Ren J, Peng Z, Yang K. A clinicopathologic multivariate analysis affecting recurrence of borderline ovarian tumors. *Gynecol Oncol* 2008;110:162–7.
11 Lenhard MS, Mitterer S, Kümper C, *et al*. Long-term follow-up after ovarian borderline tumor: relapse and survival in a large patient cohort. *Eur J Obstet Gynecol Reprod Biol* 2009;145:189–94.
12 Tinelli R, Tinelli A, Tinelli FG, *et al*. Conservative surgery for borderline ovarian tumors: a review. *Gynecol Oncol* 2006;100:185–91.
13 Kaern J, Tropé CG, Kristensen GB, *et al*. DNA ploidy; the most important prognostic factor in patients with borderline tumors of the ovary. *Int J Gynecol Cancer* 1993;3:349–58.
14 Ayhan A, Guvendag Guven ES, Guven S, *et al*. Recurrence and prognostic factors in borderline ovarian tumors. *Gynecol Oncol* 2005;98:439–45.
15 Kurman RJ, Seidman JD, Shih IM. Serous borderline tumours of the ovary. *Histopathology* 2005;47:310–5.
16 Shih I. Ovarian serous low malignant potential (borderline) tumor--does "micropapillary" matter? *Gynecol Oncol* 2010;117:1–3.
17 Katzenstein AL, Mazur MT, Morgan TE, *et al*. Proliferative serous tumors of the ovary. Histologic features and prognosis. *Am J Surg Pathol* 1978;2:339–55.
18 Bostwick DG, Tazelaar HD, Ballon SC, *et al*. Ovarian epithelial tumors of borderline malignancy. A clinical and pathologic study of 109 cases. *Cancer* 1986;58:2052–65.
19 Kominsky SL. Claudins: emerging targets for cancer therapy. *Expert Rev Mol Med* 2006;8:1–11.
20 Singh AB, Dhawan P. Claudins and cancer: Fall of the soldiers entrusted to protect the gate and keep the barrier intact. *Semin Cell Dev Biol* 2015;42:58–65.
21 Swisshelm K, Macek R, Kubbies M. Role of claudins in tumorigenesis. *Adv Drug Deliv Rev* 2005;57:919–28.
22 Knust E, Bossinger O. Composition and formation of intercellular junctions in epithelial cells. *Science* 2002;298:1955–9.
23 Morin PJ. Claudin proteins in human cancer: promising new targets for diagnosis and therapy. *Cancer Res* 2005;65:9603–6.
24 Singh AB, Sharma A, Dhawan P. Claudin family of proteins and cancer: an overview. *J Oncol* 2010;2010:1–11.
25 Kwon MJ. Emerging roles of claudins in human cancer. *Int J Mol Sci* 2013;14:18148–80.
26 McShane LM, Altman DG, Sauerbrei W, *et al*. Reporting recommendations for tumor marker prognostic studies (REMARK). *J Natl Cancer Inst* 2005;97:1180–4.
27 El-Balat A, Arsenic R, Sänger N, *et al*. Fascin-1 expression as stratification marker in borderline epithelial tumours of the ovary. *J Clin Pathol* 2016;69:142–8.
28 El-Balat A, Schmeil I, Karn T, *et al*. TFF3 Expression as Stratification Marker in Borderline Epithelial Tumors of the Ovary. *Pathol Oncol Res* 2018;24:277–82.
29 Harhaj NS, Antonetti DA. Regulation of tight junctions and loss of barrier function in pathophysiology. *Int J Biochem Cell Biol* 2004;36:1206–37.
30 Qin W, Ren Q, Liu T, *et al*. MicroRNA-155 is a novel suppressor of ovarian cancer-initiating cells that targets CLDN1. *FEBS Lett* 2013;587:1434–9.
31 Vang R, Hannibal CG, Junge J, *et al*. Long-term Behavior of Serous Borderline Tumors Subdivided Into Atypical Proliferative Tumors and Noninvasive Low-grade Carcinomas: A Population-based Clinicopathologic Study of 942 Cases. *Am J Surg Pathol* 2017;41:725–37.
32 du Bois A, Ewald-Riegler N, de Gregorio N, *et al*. Borderline tumours of the ovary: A cohort study of the Arbeitsgmeinschaft Gynäkologische Onkologie (AGO) Study Group. *Eur J Cancer* 2013;49:1905–14.
33 McKenney JK, Gilks CB, Kalloger S, *et al*. Classification of extraovarian implants in patients with ovarian serous borderline tumors (tumors of low malignant potential) based on clinical outcome. *Am J Surg Pathol* 2016;40:1155–64.
34 Kurman RJ, Shih I. The dualistic model of ovarian carcinogenesis: revisited, revised, and expanded. *Am J Pathol* 2016;186:733–47.
35 Singer G, Oldt R, Cohen Y, *et al*. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. *J Natl Cancer Inst* 2003;95:484–6.
36 Pohl G, Ho CL, Kurman RJ, *et al*. Inactivation of the mitogen-activated protein kinase pathway as a potential target-based therapy in ovarian serous tumors with KRAS or BRAF mutations. *Cancer Res* 2005;65:1994–2000.
37 Hsu CY, Bristow R, Cha MS, *et al*. Characterization of active mitogen-activated protein kinase in ovarian serous carcinomas. *Clin Cancer Res* 2004;10:6432–6.
38 Kim B, Breton S. The MAPK/ERK-Signaling Pathway Regulates the Expression and Distribution of Tight Junction Proteins in the Mouse Proximal Epididymis. *Biol Reprod* 2016;94:22.