

Claudin-5 Is Associated with Elevated TATI and CA125 Levels in Mucinous Ovarian Borderline Tumors

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Abstract. *Background/Aim:* Claudin proteins represent a large family of integral membrane proteins crucial for tight junction (TJ) formation and function and are abnormally regulated in several human cancers. The aim of the present study was to study the expression levels of claudin-5 in pre-malignant disease as borderline mucinous ovarian tumors. Previous reports have suggested that claudin-5 over-expression correlates with aggressive behaviour in serous ovarian adenocarcinoma, breast cancer and in pancreatic adenocarcinoma. *Patients and Methods:* We investigated the expression of claudin-5 in mucinous ovarian borderline tumors and its correlation with clinico-pathological parameters and the expression of serum markers cancer antigen (CA) 125 and tumor-associated trypsin inhibitor (TATI). *Results:* A total of 29 mucinous borderline tumor tissue samples were analyzed using immunohistochemical staining for claudin-5. An association between strong claudin-5 expression and higher serum levels of TATI ($p=0.04$) and CA125 ($p=0.008$) were found. There was also an association between claudin-5 expression and the presence of ascites ($p=0.02$). *Conclusion:* Changes in claudin-5 expression may play a role in malignant transformation.

Tight junctions (TJs) are critical structures for the maintenance of cellular polarity, as well as the establishment of a permeability barrier for paracellular transport in

epithelial and endothelial cells (1). The two major integral membrane constituents of tight junctions are occludin and claudin proteins (1, 2). Loss of TJ formation is believed to be crucial for the increased accessibility of the tumor cells to nutrients, increased cell proliferation and increased motility associated with metastasis (2).

Claudin proteins represent a large family of integral membrane proteins crucial for TJs. They have been shown to be up-regulated in various cancers and have been suggested as possible biomarkers and targets for cancer therapy (3, 4). There are 28 known claudins (1, 4). Claudin-5, for instance, is mainly expressed in endothelial cells in non-neoplastic tissues and takes part in forming the blood-brain barrier (BBB). In contrast to the general thought that claudin expression would decrease during tumorigenesis as TJs are lost during malignant transformation, claudin expression seems to change in a tissue-specific manner (1, 3-4).

Borderline ovarian tumors (BOTs) were first described in 1929 and further characterized by the World Health Organization (5). They comprise 10-15% of all ovarian epithelial tumors. The median age of patients with BOT is lower than the median age of patients with invasive ovarian epithelial carcinoma (5, 6). Similarly to BOTs, most cancers originate from epithelial tissues and are characterized by aberrant growth control and loss of differentiation and tissue architecture. There is usually no invasion of the surrounding stroma, although microinvasion can occur in a small portion of cases. Approximately 70% of these tumors are stage I at the time of diagnosis with a 5-year survival rate of 95-97% (5). No ideal biomarker is currently used for the management of ovarian cancer. The cancer antigen 125 (CA125) and tumor-associated trypsin inhibitor (TATI) have been used as markers for ovarian cancer (5-7).

Recent studies have implicated alterations of a number of claudin isotypes in malignancies. Claudin-5, for example, plays a role in the regulation of BBB permeability during the

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Table I. *Claudin-5 expression in mucinous ovarian borderline tumors (n=29).*

Clinical parameter	No (%) / median (range) of negative cases	No (%) / median (range) of weak or moderate positive cases	No (%) / median (range) of strong positive cases	Significance (p-value)
All cases	12 (41)	11 (39)	6 (21)	
Ascites				
No	11 (92)	9 (82)	2 (33)	
Yes	1 (8)	2 (18)	4 (67)	0.02
missing 0				
LN(CA-12-5) IU/ml (median)	3.1 (1.4-4.3)	3.3 (2.0-5.3)	4.4 (3.9-5.4)	0.008
missing 0				
TATI nmol/l (median)	1.5 (0.8-8)	12 (0.8-61)	15 (3.6-43)	0.04
missing 10				
Peritoneal cytology				
I	3 (30)	2 (18)	0 (0)	
II	7 (70)	5 (46)	3 (60)	
III	0 (0)	4 (36)	2 (40)	
IV	0 (0)	0 (0)	0 (0)	
V	0 (0)	0 (0)	0 (0)	N.S.
missing 3				

brain metastatic process (8). Strong claudin-5 expression is a poor prognostic sign in pancreatic adenocarcinoma (9). It has also been suggested that claudin-5 is involved in cancer cell motility (10).

This study was undertaken to assess the expression of claudin-5 in borderline ovarian tumor and to investigate its role in malignant transformation.

Patients and Methods

Patients and patient selection. The study population consisted of 29 mucinous borderline ovarian cancer patients treated in the Department of Obstetrics and Gynecology, Oulu University Hospital from 2001-2009. Twenty-seven out of 29 patients were stage I disease, whereas two patients presented stage IIIA disease. These two cases included mixed tumor comprising components of Brenner's tumor and intestinal mucinous borderline tumors. The other case included small intraepithelial focus of carcinoma. All patients underwent full surgical staging with pelvic and periaortic node dissection. Eleven out of 29 were intestinal mucinous ovarian borderline tumors and two cases represented endocervical mucinous borderline tumor. Almost half (14/29) were subtyped as only mucinous borderline tumors. Histological material was collected from the files of the Department of Pathology, University of Oulu. Clinical and follow-up information including age, weight, survival status, overall survival, relapse-free survival, histology and presence of ascites was collected. The serum CA125 and TATI were evaluated. The local Ethics Committee has reviewed and approved this study.

Immunohistochemistry. Tissue samples had been fixed in 10 % buffered formalin, embedded in paraffin and sectioned. Prior to the application of a primary antibody, the sections were heated in a microwave oven in 10 mmol/l citrate buffer, pH 6.0, for 10 minutes. After a 60-minute incubation with the primary antibody (mouse anti-claudin-5, clone 4C3C2, dilution 1:50; Zymed Laboratories Inc., South San Francisco, CA, USA), a biotinylated secondary anti-

rabbit antibody and a Histostain-SP kit (Zymed Laboratories Inc.) were used. The primary antibody has been previously characterized (8). The colour was developed by diaminobenzidine and, thereafter, the sections were lightly counterstained with hematoxylin and mounted with Eukitt (Kindler, Freiburg, Germany). Negative control staining was carried out by replacing the primary antibody with non-immune mouse serum and phosphate-buffered saline. Only membrane-bound immunoreactivity was considered significant. The immunostaining was assessed in four categories as follows: negative, no immunostaining present; weak positive, less than 25% of cells positive; moderate positive, 25%-50% of cells positive; and strong positive, 50% of cells positive (Figure 1).

The correlations with claudin-5 expression and clinical parameters were evaluated using the Chi-squared and Kruskal-Wallis tests. All *p*-values <0.05 were considered significant. All statistics were computed using the SPSS software, version 19 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

Results

The median age of the patients was 47 years (19-81) and their body mass index (BMI) was 26 (15-34). Ascites was present in 7 cases. Seventeen (59 %) out of 29 cases were claudin-5-positive. Strong positive staining was found in 6 out of 29 tumors (21%). There was a significant association between claudin-5 expression and the presence of ascites (*p*=0.02). At the study point, no relapse was found.

The median for preoperative CA125 was 44 IU/ml (4-216) (Table I). The serum CA125 levels were higher in claudin-5-positive samples (*p*=0.008, Figure 2a). Median preoperative TATI levels were 7 nmol/L (0.8-43) and its expression levels were higher in claudin-5-positive samples (*p*=0.04), as shown in Figure 2b.

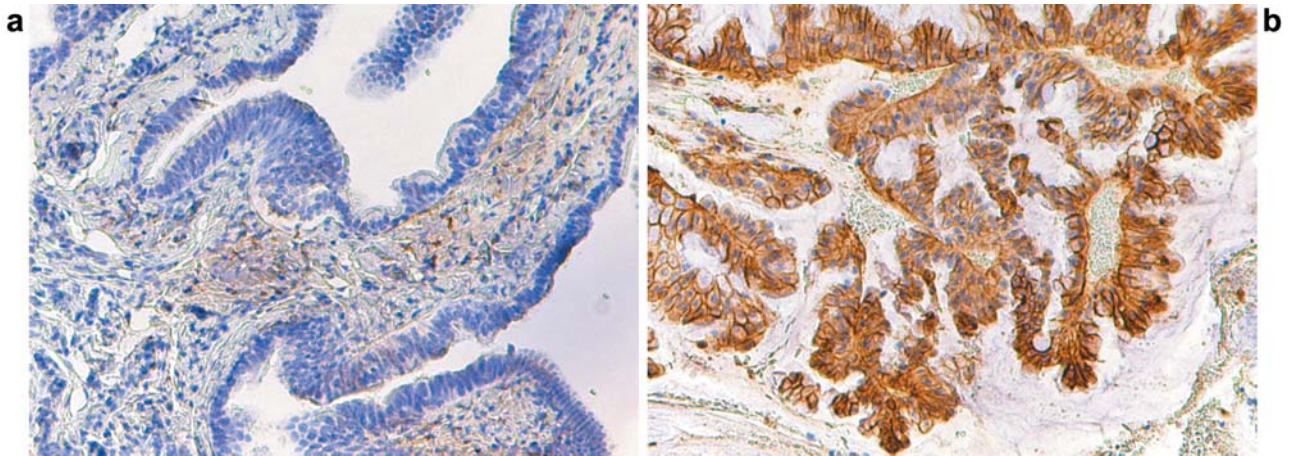


Figure 1. Negative (a) and strong positive (b) claudin-5 staining in intestinal mucinous ovarian borderline tumor.

No statistically significant differences in peritoneal cytology and claudin expression was seen, although a trend towards more intense claudin-5 expression in patients with peritoneal cytology class III is obvious.

Discussion

Tight junctions are the most apically localized part of the epithelial junctional complex. They are responsible for the formation and maintenance of the permeability barrier in polarized epithelial cells. Modification or loss of these dynamic structures contributes to disease states. An increased permeability of TJs in epithelia is an important factor in cancer progression, while TJs are typically lost in neoplasia (1-4, 9-12).

Strong claudin-5 expression is associated with the presence of ascites ($p=0.02$). Generally, claudin-5 expression is linked to a sealing function. Claudin-5 takes part in the formation of the BBB and is also expressed in podocytes, cell types that participate in glomerular filtration (13). Its expression has also been detected in mesothelial cells of pleural tissues (14). Tight junctions, the most apical structure between epithelial and endothelial cells, control the paracellular diffusion of ions and certain molecules. It has a vital role to maintain cell integrity. In metastatic brain tumors the blood-brain barrier (BBB) is disturbed and the loss of cohesion on TJs can lead to invasion and thus metastasis of cancer cells. In breast tumors, claudin-5 plays a role in the regulation of tumour cell motility and contribute to the control and loss of control of basement membrane. Patients whose tumors expressed high levels of claudin-5 had shorter survival time than those with low levels ($p=0.004$) (10). Also, strong claudin-5 expression is a poor prognostic sign in pancreatic adenocarcinoma (9).

Claudin-7 and its truncated form have been shown to influence the expression of prostate-specific antigen (PSA)

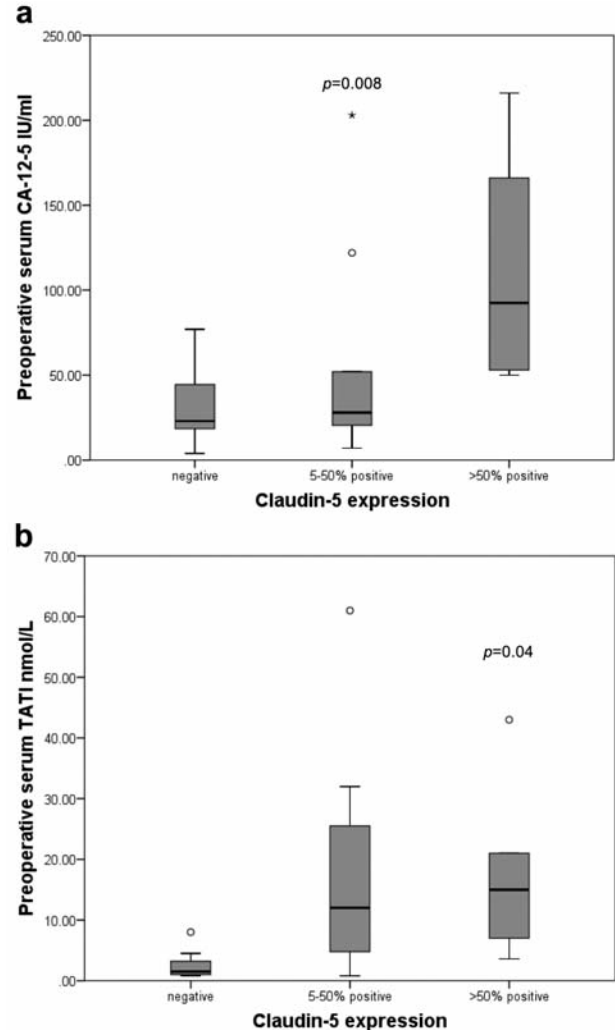


Figure 2. Serum CA12-5 levels (2a), as well as TATI (2b) levels are higher in claudin-5 positive samples.

(15-16). Lack of claudin-7, on the other hand, increases expression of cytokines, matrix metalloproteinase (MMP) 3 and 7 and cyclo-oxygenase 2 in mouse intestine (17). Response to estrogen metabolites could be one factor inducing claudin-5 over-expression in ovarian tumors. It is, thus, possible that claudin-5 in some analogous manner might influence expression or secretion of TATI and CA125. Regarding hormones, estrogen, but not progesterone, decreases the expression of claudin-5 in vascular endothelial cells in the murine uterine endometrium, although this effect appears late after the estrogen stimulation (18). On the other hand, glucocorticoids and 17-beta estradiol have been shown to increase claudin-5 expression (19).

Although the expression pattern of claudins is tissue-specific, most tissues express multiple claudins; it is possible that exact ratios of various claudin proteins determine the permeability of TJs (1-4, 7, 10, 18). For example, claudin 3, 4 and 7 are up-regulated in ovarian cancers subtypes and they promote invasion (11). Their expression is not increased in benign ovarian cystadenomas, clearly associating the presence of these proteins with malignancy (3, 4, 11-12).

This is the first study on claudin expression in mucinous ovarian borderline tumors. Even though considered an endothelial marker, claudin-5 was present in epithelial lesions. It has been previously reported that claudin-5 is strongly expressed in malignant epithelial ovarian lesions, whereas its expression is weak in benign conditions (3, 11). Claudin-5 positivity is associated with increased grade, advanced stage and poor survival of serous ovarian cancer patients. The highest intensity of claudin-5-positive samples was found in high grade and advanced stage carcinomas (3, 11).

In the present report, we show an association between strong claudin-5 expression and high serum levels of TATI ($p=0.04$) and CA 125 ($p=0.008$). Increased claudin-5 expression may associate with disruption of TJs and cell transformation. Claudin-5 expression in tumors, on the other hand, appears only to be a sign of tumor aggressivity thus indirectly affecting permeability of peritoneal cells by inducing inflammation and ascites, which could account for the observation reported in this study.

Strong claudin-5 expression is a poor prognostic sign in pancreatic adenocarcinoma. Claudin-5 is also involved in breast cancer motility and could be a contributing factor to metastatic disease in human breast cancer. We conclude that up-regulation in claudin-5 expression may associate with cell transformation in borderline ovarian tumors.

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