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Claudin proteins in ovarian cancer

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Abstract. Members of the claudin family of tight junction proteins have been found altered in several malignancies, including ovarian cancer. Because claudin-3 and -4 are elevated in the vast majority of ovarian tumors, they may represent useful biomarkers for detection and prognosis, as well as ideal targets for therapy using the *Clostridium perfringens* enterotoxin.

1. Introduction

Gene expression profiling approaches have allowed large-scale unbiased analysis of gene expression in cancer. These methods thus represent ideal approaches for the identification of novel biomarkers for detection, diagnosis, and prognosis of cancer. For example, in ovarian cancer, early gene expression profiling studies identified HE4 (WFDC2) as a promising marker for screening of this disease [1,2]. One of these early studies also identified two claudin genes, *CLDN3* and *CLDN4* as highly up-regulated in ovarian cancer [2]. Work from several groups has now confirmed the overexpression of these proteins in ovarian cancer, and these findings may have significant implications for detection, prognosis and therapy of this disease [3]. In addition, a number of claudin genes, including *CLDN1,3,4,5,7,10,16,18*, have been found altered in various other human cancers [3]. In this chapter, I will review the latest findings on claudin expression and function in ovarian cancer, as well as the clinical significance of these findings.

2. Tight junctions and claudin proteins

The claudin proteins represent a family of at least 21 closely related members expressed in a wide variety of normal and neoplastic tissues [4–6]. The claudin proteins are membranes proteins which play crucial roles in tight junction (TJ) formation and function [5]. TJs are critical structures in the establishment of a permeability barrier for paracellular transport in epithelial and endothelial cells. TJs are also believed to play important roles in the maintenance of cell polarity by providing a "fence" between the apical and basolateral sides of the cell [7]. Claudins constitute the backbone of the TJs and interact with each other in homotypic and heterotypic fashion. Normal tissues typically express multiple claudins, but the expression pattern of claudins is tissue-specific and can vary considerably among various tissues [5,6]. The exact combination of claudins present in a given tissue is believed to be one of the factors that determine the properties of the TJ, including TJ strength and paracellular selectivity.

Claudins have two transmembrane domains and with both the N- C-termini ends of the protein located in the cytoplasm (see Fig. 1). This arrangement results in the formation of two extracellular loops, which can interact with extracellular components. The C-terminal region contains a PDZ-domain binding motif that can potentially interact with PDZ-domain containing proteins such as the ZO proteins. A number of proteins such as PKC and PTEN have been shown to interact directly or indirectly with the TJs suggesting an important signaling component for these structures [5].

Tumor cells typically exhibit structural and functional deficiencies in their TJs [8]. These deficiencies are associated with a loss of polarity and differentiation. Loss of TJ integrity may be important to allow the nutrients and other necessary factors to penetrate the tumor mass [9]. In addition, the loss of polarity, differentiation, and adhesive properties associated with the TJs may be crucial in acquiring a metastatic phenotype [10].

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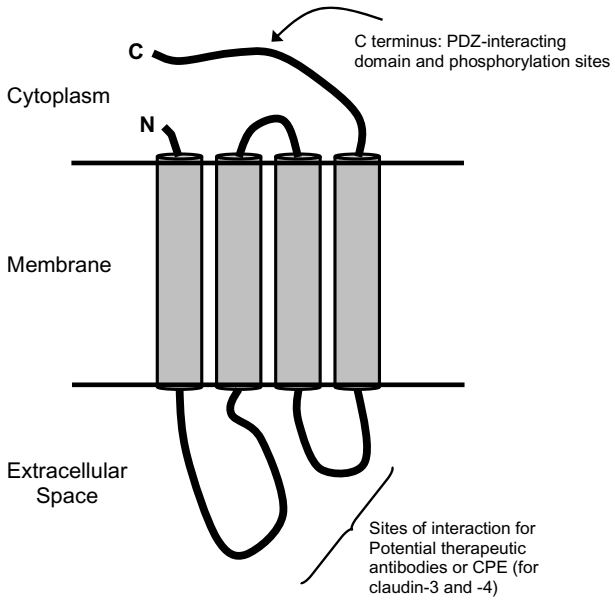

Fig. 1. Schematic structure of claudin proteins. The functionally relevant regions are indicated.

3. Claudins in ovarian cancer

Serial Analysis of Gene Expression (SAGE) was among the first high-throughput techniques to be used to analyze ovarian cancer gene expression [2] and both *CLDN3* and *CLDN4* were found among the genes most highly up-regulated in this cancer. Follow-up studies using Affymetrix arrays, and various other techniques have validated these findings and extended the observation to large numbers of samples of various stages, grades, and subtypes [6,11–19] (see Table 1). Interestingly, a few studies have observed overexpression of *CLDN7* in ovarian cancer [6,13,17], although this change is less consistent than what is observed for *CLDN3* and *CLDN4* . Overexpression of claudins appears to be most prominent in epithelial tumors, since sex-cord and stromal tumors do not express these proteins at appreciable levels [19]. Interestingly, *in silico* analysis of the expression of all the known claudin genes suggest that *CLDN3*, *CLDN4*, and *CLDN7* may be coordinately regulated [6], although the mechanisms regulating their expression have not been elucidated. Additionally, another claudin gene, *CLDN16*, has also been suggested to be elevated in ovarian cancer [20].

When examined at the protein level, it is found that claudin-3 and claudin-4 proteins are elevated in up to 90% of the ovarian cancer samples examined [11,16]. Interestingly, claudin proteins can frequently be detected in the cytoplasm of ovarian tumors [2,11,12], suggesting that claudins in these cancers may not participate in TJ formation, but may have other roles in tumorigenesis [3,21]. The mechanisms regulating claudin localization are not well understood, but likely involve phosphorylation [22–25]. The mislocalization of claudin proteins in cancer cells may have important significance in the tumorigenesis process.

4. Claudins as biomarkers

Because of their expression patterns, claudin proteins may represent useful ovarian cancer markers for early detection, diagnosis, or therapy. Indeed, a recent study using recursive descent partition analysis, demonstrated that *CLDN3* expression was a robust marker to distinguish serous, endometrioid and clear cell from the normal ovarian surface epithelium and mucinous tumors [16]. Further partition with VEGF expression patterns allowed the complete separation of the tumors from the normal cells. At this time, it is unclear whether claudin proteins are shed into the bloodstream and found in the serum of ovarian cancer patients, making this discussion mostly academic. Moreover, initial expression data suggest that claudin expression may not have a sufficient specificity and sensitivity for ovarian cancer detection in the general population, although combination of markers, including claudins, may represent an attractive possibility for early detection of ovarian cancer [15,16,26]. For example, it has been shown that every ovarian tumor studied that

Table 1
Studies of claudin expression in ovarian cancer

Refs	Techniques used	Findings in ovarian cancer
[2]	SAGE, IHC	*CLDN3*, *CLDN4* up
[11]	RT-PCR, IHC	*CLDN3*, *CLDN4* up
[20]	RT-PCR	*CLDN16* up
[12]	IF, Immunoblotting	*CLDN3*, 4 up
[13]	Human Affy GeneChip (U95Av2), qRT-PCR	*CLDN3*,4, 7 up
[14]	Human Affy GeneChip Hu95, IHC	*CLDN4* up
[15]	Human Affy GeneChip Hu03, IHC, *in silico* analysis	*CLDN3* up
[16]	Human Affy GeneChip U95, IHC (for *CLDN3*)	*CLDN3*, *CLDN4* up
[6]	*In silico* analysis, qRT-PCR	*CLDN3,4,7* up
[17]	Human Affy GeneChip U133A, qRT-PCR	*CLDN3,4,7* up
[18]	Proteomics (HPLC-ESI-MS/MS)	Claudin-4 up in drug resistance
[19]	IHC	Claudins expressed in epithelial tumors

lacks CA125 expression expresses claudin-3, suggesting that this claudin may represent a useful complementary marker to complement CA125 in the detection of ovarian cancer [26].

Claudin-3 expression was found associated with poor outcome in ovarian cancer [15], although another study found that claudin-3 expression (but not claudin-4) was associated with higher grade ovarian tumors [12]. Additional work will be necessary to clarify whether claudin-3 can be a predictor of outcome or whether it is associated with other known clinicopathological parameters. In another study, no significant differences could be detected in claudin-4 expression in primary and metastatic tissues [14].

Claudins may also represent useful biomarkers in differential diagnosis. For example, a recent report has shown that diffuse malignant peritoneal mesothelioma (DMPM) and ovarian cancer, which are very similar histologically, could be distinguished at the molecular level with a small number of markers that included several claudins [27]. Claudins-3,-4, and -6 were found elevated in ovarian cancer compared to DMPM while claudin-15 was found to be higher in DMPM. Such differential diagnosis may be crucial in tailoring optimal therapy for patients with closely related tumor types.

5. Claudin function in cancer

While claudin overexpression of claudins in ovarian and other cancers is a well-accepted observation, the exact role of these proteins in tumorigenesis is a matter of debate. Since cancer cells typically exhibit deficiencies in TJs, the loss of certain claudins in various cancers has been interpreted as a mechanism for the dismantlement of the TJ and increased invasiveness [28–30]. Overexpression of TJ proteins, however, as is observed in ovarian cancer for claudin-3, claudin-4, is more difficult to explain. It might be hypothesized that inappropriate claudin overexpression may somehow lead to the destabilization of TJs. However, there is no evidence for such a mechanism. As mentioned earlier, while claudin proteins have well-known roles in TJ formation and function, they may also have additional roles in signaling [5]. These signaling functions may take a dominant role in cancer. Agarwal et al. found that claudin-3 and claudin-4 overexpression is associated with increased motility, invasion, and survival, suggesting a role for these proteins in ovarian cancer metastasis [21]. The increased invasiveness may be due, at least in part, to the activation of MMPs. Interestingly, there is ample evidence for the activation of MMPs by claudins in other systems [31–33]. There is a distinct possibility that claudin overexpression in cancer may have a role in EMT. However, these activities may be cell- and claudin-specific as overexpression claudin-4 in pancreatic cancer cells was shown to reduce invasiveness [34], and claudin-1 expression has been observed to increase apoptosis in three-dimensional cultures of breast cancer cells [35].

6. Claudin in ovarian cancer therapy

Because claudin proteins have two relatively large extracellular loops (see Figure), they have been suggested as possible targets for antibody-directed therapy. Initial studies have shown that anti-claudin antibodies can bind to the surface of the cells expressing the respective claudin proteins, and could therefore potentially be used to specifically deliver cytotoxic agents to these claudin-expressing cancer cells [36]. In the case of ovarian cancer, claudin-3,-4,-7, and possibly 16, may be worth investigating as potential targets.

Claudin-3 and claudin-4 are the natural receptors for the *Clostridium perfringens* enterotoxin (CPE) [37].

Binding of CPE to these claudins results in rapid lysis of the cells, mediated by the formation of a large multiprotein membrane pore complex, and inappropriate regulation of ion transport. Cells that do not express claudin-3 or claudin-4 are not susceptible to CPE toxicity. Therefore, the overexpression of these claudins in various tumors suggests the use of CPE as a chemotherapeutic agent, and early experiments were successful at demonstrating a proof-of-principle for this idea [38,39]. Interestingly, Santin et al. recently found that CPE was highly effective at killing fresh ovarian cancer cells *in vitro* and in SCID mice, regardless of their drug resistance status, suggesting a very promising approach for the treatment of patients with drug-resistant ovarian cancer [40]. Importantly, claudins do not appear to be generally down-regulated in drug-resistant ovarian cancer and claudin-4 may actually be increased [18]. Ovarian cancer may represent an ideal candidate for CPE therapy, as it typically remains confined to the peritoneal cavity and IP administration of CPE may circumvent toxicity that may be encountered with systemic administration of this toxin. As mentioned above, CPE therapy may be particularly useful in the treatment of drug-resistant tumors as the mode of killing appears to be completely different than that of conventional chemotherapy. On the other hand, a few limitations may reduce the effectiveness of CPE therapy for ovarian cancer. First, some cancers appear to express mostly cytoplasmic claudin-3 and claudin-4 proteins. Second, it is unclear whether CPE could distribute uniformly throughout the abdominal cavity and penetrate the tumor masses. Third, repeated CPE administration may lead to an immune response against this protein. Finally, despite local administration, CPE may still exhibit toxicity. In spite of these limitations, preclinical investigations of this approach have been extremely promising and clinical trials will be crucial in determining the feasibility of this strategy.

7. Conclusions

Claudin proteins have been found differentially expressed in a large number of cancers. In ovarian cancer, claudin-3, -4 may represent novel biomarkers for the detection and/or diagnosis of the disease. In addition, the expression of these proteins on the surface of most ovarian cancer cells suggest novel therapeutic strategies using CPE, a toxin that specifically kills cell expressing these proteins. Considering the difficulties surrounding current ovarian cancer therapy, it will be important to aggressively pursue alternative treatment strategies for this disease.

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