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Claudin Proteins in Human Cancer: Promising New Targets for Diagnosis and Therapy

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Claudin Proteins in Human Cancer: Promising New Targets for Diagnosis and Therapy

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Abstract

The tight junction proteins claudins are abnormally regulated in several human cancers. In particular, claudin-3 and claudin-4 are frequently overexpressed in several neoplasias, including ovarian, breast, pancreatic, and prostate cancers. Although the exact roles of these proteins in tumorigenesis are still being uncovered, it is clear that they represent promising targets for cancer detection, diagnosis, and therapy. (Cancer Res 2005; 65(21): 9603-6)

Claudins Are Tight Junction Proteins

Tight junctions, together with adherens junctions and desmosomes, form the apical junctional complex in epithelial and endothelial cellular sheets. Adherens junctions and desmosomes are responsible for the mechanical adhesion between adjacent cells, whereas tight junctions are essential for the tight sealing of the cellular sheets, thus controlling paracellular ion flux and therefore maintaining tissue homeostasis (1). Tight junctions also play a crucial role in the maintenance of cell polarity by forming a fence that prevents lateral diffusion of membrane proteins and lipids, thereby maintaining the differential composition of the apical and basolateral domains. Finally, because of the ability of tight junction proteins to recruit signaling proteins (2), tight junctions have also been hypothesized to be involved in the regulation of proliferation, differentiation, and other cellular functions.

When observed by electron microscopy, tight junctions form multiple strands that seem to provide the structural basis for adhesion between adjacent cells (1). Tight junctions are composed of three major integral membrane proteins, occludin, claudins, and junctional adhesion molecules. Although the exact roles of these proteins are not completely clear, it seems that the claudins form the backbone of the tight junction strands. The claudin family of proteins is comprised of 23 members of closely related transmembrane proteins (see Fig. 1). Although the expression pattern of claudins is tissue specific, most tissues express multiple claudins, which can interact in both homotypic and heterotypic fashion to form the tight junction strands. It is believed that the exact combination of claudin proteins within a given tissue can determine the selectivity and strength of the tight junctions. Claudins are polymerized together within a given cell and can interact with the claudin of the adjacent cells to form an adhesive structure.

The high degree of cellular organization typically observed in normal differentiated tissues is often lost in cancer. Tumor cells frequently exhibit abnormal tight junction function as well as decreased differentiation and cell polarity (3, 4). Loss of tight junction integrity may be particularly important in allowing the diffusion of nutrients and other factors necessary for the survival and growth of the tumor cells (5). In addition, decreased polarity and differentiation may be important for the metastatic phenotype, where individual cells must leave the primary site and enter the blood vessels to reach distant sites (6). Finally, the destruction of functional tight junctions in cancer may have a role in growth control. For example, in *Drosophila*, mutations in many tumor suppressor genes lead to loss of cell polarity and overproliferation of the epithelia (7). Based on the similarity between the vertebrate and *Drosophila* epithelia, mammalian cells are likely to require cytoarchitectural cues for cell growth control as well.

Claudin Expression in Cancer

The expression of occludin and claudins, the two major transmembrane proteins that contribute to formation of tight junctions, has been found to be altered in several cancers. An early study in the field showed that occludin was often down-regulated in gastrointestinal tumors (8). Similarly, other studies have shown that claudins are down-regulated in various cancers. For example, claudin-1 has been found to be reduced in breast cancer (9, 10) as well as in colon cancer (11). Claudin-7 has also been found down-regulated in invasive breast cancer (12) and in head and neck cancer (13). These reports of decreased tight junction protein expression in cancer are consistent with the generally accepted idea that tumorigenesis is accompanied by a disruption of tight junctions, a process that may play an important role in the loss of cohesion, invasiveness, and lack of differentiation observed in cancer cells. In addition to the down-regulation of protein levels, phosphorylation of tight junction proteins, including claudins, may affect tight junction function in cancer (14). For example, phosphorylation of claudin-1 by mitogen activated protein kinases (15) and protein kinase C (16), as well as phosphorylation of claudin-5 by cyclic AMP–dependent protein kinase (17, 18) have been reported. Also, WNK4 kinase has been shown to phosphorylate claudin-3 and claudin-4, and decrease tight junction function (19). Interestingly, phosphorylation of claudin-3 and claudin-4 in ovarian cancer cells has been shown to disrupt tight junctions (20).

Paradoxically, other studies have shown that certain claudin proteins are up-regulated in cancer. In fact, the overwhelming majority of the studies published thus far report an over-expression of claudins in various cancers (see Table 1). One of the first studies reporting this fact was a serial analysis of gene expression (SAGE) study of ovarian cancer showing that *CLDN3* and *CLDN4* (encoding claudin-3 and claudin-4, respectively) were among the most highly up-regulated genes in this cancer (21). Several additional reports have since confirmed the high

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expression of these two claudins in ovarian cancer (22–25). In addition, claudin-3 and claudin-4 have also been reported to be expressed in other cancers, such as breast (26), prostate (27), and pancreatic (28–32) cancers. Other claudins are differentially expressed in a number of human neoplasms and these data are summarized in Table 1.

Roles of Claudin in Cancer

As mentioned above, the loss of claudins and other tight junction proteins in cancer has been interpreted as a mechanism for the loss of cell adhesion and an important step in the progression of cancer to metastasis. Consistent with this hypothesis, a recent study showed that expression of claudin-4 in pancreatic cancer cells reduces invasiveness of these cells (33). In addition, claudin-1 reexpression in cancer cells can lead to increased apoptosis in three-dimensional cultures (34). On the other hand, as discussed previously, many claudins, such as claudin-3 and claudin-4, are typically up-regulated in many cancers (Table 1), suggesting that these proteins may have a positive effect on tumorigenesis. Recent work has shown that, at least in the case of ovarian cells, expression of claudin-3 and claudin-4 may lead to an increase in invasion, motility, and cell survival (35), all characteristics important for metastasis. Consistent with these *in vitro* findings is a report that claudin-4 expression in pancreatic intraductal papillary mucinous neoplasms was associated with a more invasive phenotype (31). Similarly, expression of claudin-3 and claudin-4 was observed in advanced ovarian cancer but not in ovarian cystadenomas (22). Therefore, the functions of claudins may be highly tissue specific and may depend on the exact molecular circuitry of the cell.

Claudins as Diagnosis Markers and Therapeutic Targets

Because of the high specificity of claudin expression patterns in cancer, it has been suggested that claudins may represent useful molecular markers for many different cancers. For example, a set of four markers, including claudin-3, was found to be sufficient to accurately identify all 158 ovarian cancers tested, including eight early-stage serous cancers (24). In addition, claudin expression may be used as a prognostic indicator because low claudin-1 expression has been shown to be associated with a poor prognosis in stage II colon cancer (11). Claudin-10 expression has also been shown to be an independent prognostic factor for hepatocellular carcinoma recurrence after curative hepatectomy (36).

Interestingly, claudin-3 and claudin-4 are receptors for the *Clostridium perfringens* enterotoxin (CPE; ref. 37). CPE is a single

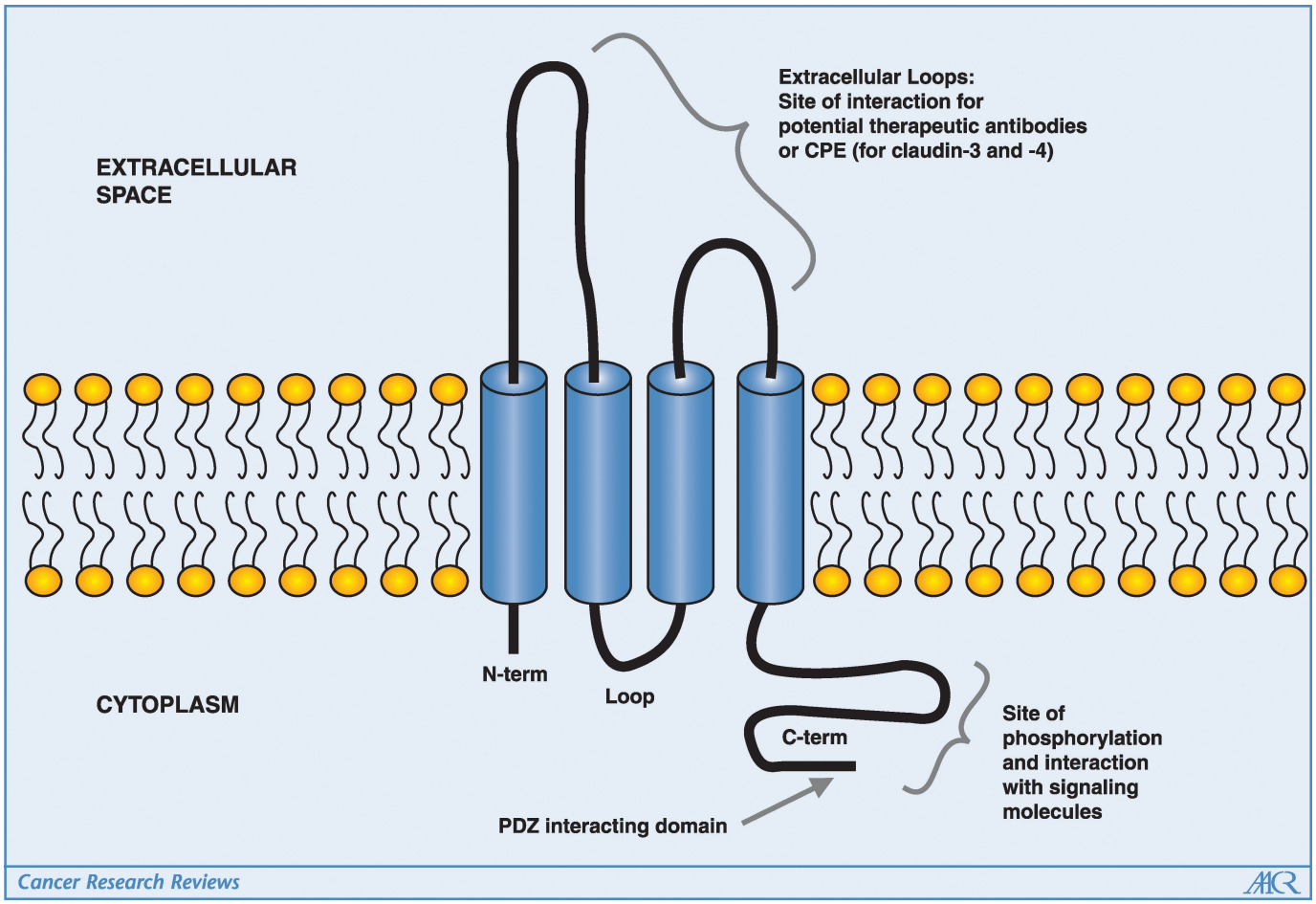

Figure 1. Structure of claudins. Claudin proteins are a family of proteins containing four transmembrane domains. The extracellular loops represent promising targets for therapy using monoclonal antibodies or, in the case of claudin-3 and claudin-4, the CPE. The COOH-terminal region of claudins is phosphorylated, contains a PDZ-binding domain, and has been implicated in signal transduction.

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Table 1. Claudin expression in cancer

Cancer	Claudin gene	Expression	References
Breast	*CLDN1*	Down	(9)
	CLDN7	Down	(12)
	CLDN1, CLDN3, CLDN4	Variable	(10)
	CLDN3	Up	(26)
	CLDN4	Up	(26)
Breast and Paget's disease	*CLDN2, CLDN3, CLDN4, CLDN5*	Variable	(44)
Colon	*CLDN1*	Variable	(11)
Gastric	*CLDN4*	Down	(45)
Hepatocellular carcinoma	*CLDN10*	Up	(36)
Hepatocellular carcinoma (mouse model)	*Cldn7*	Up	(46)
Head and neck squamous cell carcinoma	*CLDN7*	Down	(13)
Squamous cell carcinoma	*CLDN1*	Up	(47)
	CLDN4	Up	(47)
Ovarian	*CLDN3*	Up	(21, 24, 25, 48)
	CLDN4	Up	(21, 23, 25)
	CLDN16	Up	(49)
Pancreatic	*CLDN4*	Up	(28–30, 32)
Pancreatic (intraductal papillary mucinous neoplasms)	*CLDN4*	Up	(31)
Prostate	*CLDN3*	Up	(27)
	CLDN4	Up	(27)
Thyroid papillary cancer	*CLDN10*	Up	(50)

polypeptide of 35 kDa, which, upon binding to its receptors, causes cytolysis through its effects on membrane permeability. High expression of claudin-3 and claudin-4 in multiple cancers may thus represent a unique opportunity for innovative therapy using CPE (38). Prostate adenocarcinoma cells expressing claudin-3 and claudin-4 have indeed been shown to be sensitive to CPE-mediated cytolysis (27). Specificity was evident as prostate cancer cells lacking claudin-3 and claudin-4 were unaffected by CPE treatment. Similar experiments established that breast (26), ovarian (39), and pancreatic (29) cancer cells are also sensitive to CPE treatment, provided that they express claudin-3 and/or claudin-4, as these cancers often do. Interestingly, human tumors grown as xenografts in immunocompromised mice could also successfully be treated using CPE, again on the condition of claudin-3 or claudin-4 expression (26, 29, 39). Importantly, these studies showed that no significant toxicity was encountered in mice upon intratumoral CPE treatment. However, claudin-3 and/or claudin-4 are expressed in several normal human tissues, including the gut, the lungs, and the kidneys (27). This expression pattern may represent a problem in the use of CPE for systemic cancer therapy, and it remains to be seen whether this approach will be useful in the clinic. Clearly, approaches that would involve regional application of CPE would be preferable. In addition, it has been suggested that a nontoxic, but claudin-specific, COOH-terminal CPE fragment (C-CPE; ref. 40) could be delivered locally to certain normal tissues and prevent CPE toxicity. Other potential problems with the use of CPE in tumor treatment include the occasional lack of surface claudin expression (22), the possibility of an immune response against CPE in treated patients as well as the penetration of CPE into the tumor mass. Additional studies will be required to clearly ascertain these issues.

The C-CPE fragment represents another potential opportunity for the treatment of claudin-3- and claudin-4-expressing tumors. Indeed, C-CPE could be used as a specific carrier for cytotoxic agents and, therefore, provide selective drug delivery. Additionally, it has been suggested that, because C-CPE can destroy tight junctions (41), this peptide may be useful in combination therapy with conventional chemotherapeutic by increasing drug delivery to the interior of tumors. However, it seems that claudin-3 and claudin-4 expression is not necessarily associated with the formation of functional tight junctions in tumors and this approach may not be generally viable (22). Because claudins are transmembrane proteins and typically have two relatively large extracellular loops (see Fig. 1; ref. 42), these proteins may also offer promising targets for antibody-based therapy. Antibodies that specifically recognize different extracellular loops have been produced and shown to specifically bind claudins on the surface of the cell, providing a proof of principle for the approach (42).

The advent of gene expression profiling techniques has allowed the unbiased identification of genes that are differentially expressed in cancer. Although tight junction proteins have been studied for their role in tumorigenesis for many years, SAGE studies of breast (43) and ovarian (21) cancers allowed for the first time the identification of specific claudin family members as potential biomarkers for these cancers. Subsequent array analyses have confirmed these findings and also identified claudins as proteins frequently altered in cancer (see Table 1). These findings are important because the unusual expression patterns of claudins suggest utility for detection, diagnosis, and treatment of drug-resistant cancers. Although clinical trials will be required to establish this potential, basic research on claudins is likely to remain valuable for providing important insights into normal and neoplastic cellular physiology.

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