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Review

Claudins in human cancer: A review

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Summary. Claudins are tight junction proteins that are critical for the sealing of cellular sheets and controlling paracellular ion flux. The claudin family of proteins is composed of at least 24 closely related transmembrane proteins, most of them are well characterized at the gene and protein levels. The claudins are present in variety of normal tissues, hyperplastic conditions, benign neoplasms, and cancers that exhibit epithelial differentiation. Loss of claudins expression has also been reported in several malignancies as well. Differential expression of various members of the claudins family in cancers can be used in confirming the histologic identity of certain cancers and excluding others. Examples include the use of immunohistochemical detection of claudins to differentiate between oncocytoma and chromophobe renal cell carcinoma, endometrial endometrioid carcinoma and seropapillary carcinoma, mesothelioma and metastatic adenocarcinoma, hepatocellular and biliary tract carcinomas, and between intestinal-type and diffuse-type gastric carcinoma. Expression of certain claudins can also be used as markers that can predict patient's prognosis. Thus, it seems that attempts to identify expression claudins in cancers are becoming increasingly useful in histologic diagnosis of tumors as well as means to assess patient's prognosis.

Key words: Claudins, Immunohistochemistry, Cancer

Introduction

Claudins are tight junction proteins which, along with adherens junctions and desmosomes form cellular sheets. Tight junctions (TJs) are critical for the sealing of cellular sheets, thereby controlling paracellular ion flux. In addition to the above, tight junctions also play critical roles in maintaining cell polarity and signal transductions (Tsukita and Furuse, 2000; van Itallie and Anderson, 2006). 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, evidence suggests that claudins form the backbone of the tight junction strands. The claudin family of proteins is composed of at least 24 closely related transmembrane proteins. Tissues usually express multiple claudins which interact with nearby claudins in the same cell and in neighboring cells to form homo- and hetero-dimers, creating the tight junction strands (Tsukita and Furuse, 2000; van Itallie and Anderson, 2006). The high degree of cellular organization typically observed in normally differentiated tissues is often lost in cancer. Tumor cells frequently show abnormal TJ function as well as decreased differentiation and cell polarity (Soler et al., 1993; Hewitt et al., 2006). Loss of epithelial integrity with change in claudins levels and resultant increased para-cellular leakage plays a critical role in providing a space for tumor cell mobility and increased nutrients' supply for tumor cells.

The TJ sealing strength varies over five orders of magnitude in different epithelia, from leaky proximal tubules to almost hermetic colon and urinary bladder. Tightness and level of claudins expressions can also change in the same epithelium according to physiological and pathological conditions, and in response to pharmacological changes (Balda et al., 1991). When MDCK cell lines are treated with human epidermal growth factor (EGF), its levels and staining of claudin-1 and claudin-2 are decreased, and levels of claudin- 4 and -7 are increased, without change of these proteins' locations. Other TJ components, such as ZO-1, ZO-2, ZO-3, and occludin do not seem to be changed by these treatments (Flores-Benítez et al., 2007). The degree of sealing of the TJ is reflected by the overall electrical resistance of an epithelium (TER). EGF has been shown to increase the TER in canine mucosal cells,

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LLC-1 PK cells, and Alveolar Epithelial Cells (AEC) (Saladik et al., 1995; Borok et al., 1996; Chen et al., 2001). In AEC, EGF stimulates the expression of claudin-4 and -7 and down-regulates the expression of claudin-3 and -5. Physiological regulations are seldom, if ever, due to a single factor displacing a parameter in one direction, but EGF plays a pivotal role in the adjustment of the permeability of TJs to physiological requirements, pathological conditions, and pharmacological interventions (Flores-Benítez et al., 2007).

Claudins expression in cancer

Recent gene and protein expression profiling analyses have shown that claudins' expression is frequently altered in several cancers (Swisshelm et al., 2005; Hewitt et al., 2006). Several studies on cancers have revealed down-regulation of claudins' expression including claudin 1 in breast cancer (Krämer et al., 2000) and claudin 7 in invasive breast cancer and in head and neck cancer (Al Moustafa et al., 2002; Kominsky et al., 2003). It is logical to expect this down-regulation of claudins, because tumorigenesis is accompanied by disruption of tight junctions, with resultant loss of cohesion, invasiveness and lack of normal process of differentiation. Down-regulation of claudins is also noted in poorly differentiated malignancies, in contrast to well-differentiated tumors that exhibit a claudin profile that closely resembles normal tissues (Fig 1A,B). However, it is interesting to note that numerous other studies have shown up-regulation of these proteins in other cancers including, as an example, claudins 3 and 4 over-expression in ovarian, breast, and prostate cancers (Long et al., 2001; Kominsky et al., 2003; Rangel et al., 2003). The over-expression of claudins in these cancers, which typically lose their TJs, is unexpected but probably related to roles unrelated to TJ formation (Hewitt et al., 2006). While the exact functions of claudins in cancer cells are not fully understood, recent work suggests that claudins are involved in survival and invasion of cancer cells (Michl et al., 2003; Agarwal et al., 2005; Morin, 2005).

In addition to their pathophysiologic functions, claudin proteins expression may have significant clinical relevance (Morin, 2005; Swisshelm et al., 2005). For example claudin 10 expression in hepatocellular carcinoma and claudin 1 in colorectal carcinoma have shown to be of prognostic value (Cheung et al., 2005; Dhawan et al., 2005). A brief review of the literature on the current research on claudins and their expression in various human cancers is detailed as follows and summarized in tables 1 and 2.

Breast cancer

Claudins display altered levels of expression in different types of breast cancer (Nacht et al., 1999; Soini, 2004; Tokes et al., 2005; Morohashi et al., 2007; Kulka et al., 2009). Gene expression and western blot analysis of breast carcinomas showed overexpression of claudin 3 and claudin 4 at the mRNA and protein levels compared to normal human mammary epithelial cells (Nacht et al., 1999). In agreement with these results, an immunohistochemical analysis of 10 primary breast carcinoma cases of varying histological grades confirmed detection of claudin 3 and 4 in all primary breast carcinomas tested, which was higher in 3/10 and 6/10 primary breast carcinomas, respectively, compared with normal mammary epithelium (Kominsky et al., 2004). Other studies revealed increased expression of claudin 4 in basal-like breast carcinomas and decreased

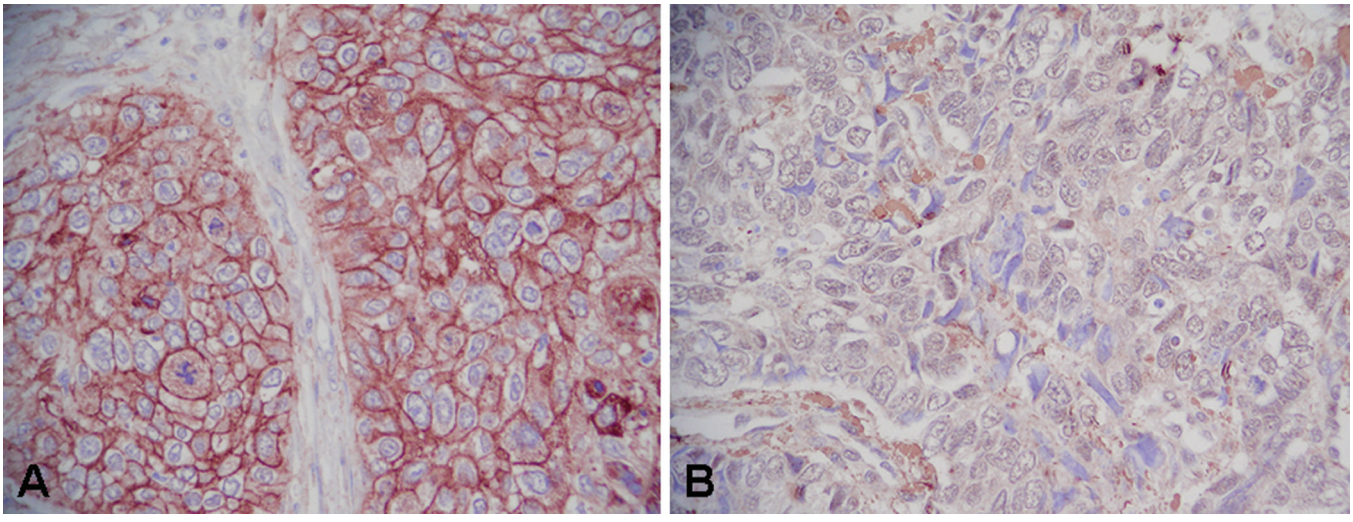

Fig. 1. A Membranous expression of claudin 1 in well-differentiated squamous cell carcinoma of the head and neck (unpublished data). **B.** Loss of claudin 1 expression in poorly differentiated squamous cell carcinoma of the head and neck (unpublished data).

expression in low grade tumors (Tokes et al., 2005; Kulka et al., 2009). Other claudins expressed in breast cancer include claudins 2 and 5 which were more often found in ductal carcinoma than lobular carcinomas (Soini, 2004).

In contrast, expression of claudin 1 and claudin 7 at the molecular level was lost or down-regulated in breast carcinomas (Krämer et al., 2000; Kominsky et al., 2003; Tokes et al., 2005). Using immunohistochemistry (IHC), Claudin 7 expression was found to be lost in the majority of high-grade invasive ductal carcinomas and high grade ductal carcinoma *in-situ*. Loss of claudin 7 expression was most likely to be associated with nodal metastases (Kominsky et al., 2003). Decreased claudin 1 expression was also found in recurrent carcinoma compared to non-recurrent carcinoma groups. Lymph node metastases from breast carcinoma also had decreased expression of claudin 1 (Morohashi et al., 2007).

Ovarian Cancer

Gene analysis on claudins reveals genes differentially expressed in ovarian cancer. Among the top six novel differentially expressed genes were two members of the claudin family of tight junction proteins. Claudin 4 and claudin 3 were up-regulated >80 folds in primary ovarian tumors compared with nonmalignant ovarian samples. The results were validated with IHC which showed strong membranous staining for claudin 3 and claudin 4, limited to the tumor component of the specimen (Hough et al., 2000). Over-expression of claudin 3 was identified all serous, endometroid, and clear cell carcinomas examined and was significantly increased in moderately and poorly differentiated tumors compared to well-differentiated tumors (Zhu et al., 2006). Expression of claudin 3 and claudin 4 enhances ovarian cancer invasion and is associated with increased matrix metalloproteinase-2 activity (Agarwal et al., 2005). Strong expression of other claudins including claudins 1,5 and 7 is also seen in a variety of benign and malignant epithelial ovarian tumors and in the epithelial component of immature teratomas (Soini and Talvensaari-Mattila, 2006). Claudins' expression was not seen in sex-cord stromal tumors or in dysgerminomas (Zhu et al., 2006). Expression of claudins was also higher in ovarian cancer effusions compared to primary carcinomas and solid metastasis and was associated with poor overall survival (Kleinberg et al., 2008). Claudin 16 expression in ovarian cancers was also identified using SAGE gene analysis, northern blot analysis and real-time PCR. Claudin 16 was upregulated in serous, clear cell, endometrioid, and mucinous ovarian cancers (Rangel et al., 2003).

Esophagus and gastric carcinomas

Claudin 1 expression was identified by IHC in approximately 80% of esophageal cancers and is lost in 20% of cases. Decrease or loss of claudin 1 expression was associated with recurrence (Miyamoto et al., 2008). Claudins 3 and 5 had more expression in adenocarcinomas versus squamous cell carcinomas. Loss of claudin 3 expression was significantly associated with presence of distant metastasis (Takala et al., 2007).

Claudins 1, 3, 4 and 5 were all expressed in gastric adenocarcinoma (Soini et al., 2006a). Claudin 4 was expressed in 53.1% of gastric carcinomas while claudin 2 in only 2.1% (Soini et al., 2006a; Aung et al., 2006). Expression of claudin 3 was found to be associated with better prognosis. Loss of claudin expression was also found to be associated more with diffuse type of gastric carcinoma, than the intestinal type (Soini et al., 2006a). As an example, the levels of claudin 4 protein and

Table 1. Common carcinomas that display expression of claudins.

Malignancy	Types of claudins expressed
Breast Carcinoma	3, 4
Ovarian surface epithelial carcinomas	1, 3, 4, 5, 7
Gastric adenocarcinoma, intestinal type	1,3, 4, 5
Colorectal carcinoma	1, 12
Hepatocellular carcinoma	7
Hepatoblastoma, fetal type	1, 2
Biliary tract carcinoma	4
Pancreatic carcinoma	1, 4
Renal cell carcinoma	1,3, 4
Chromophobe renal cell carcinoma	7
Oncocytoma	8
Prostate carcinoma	1,3, 4, 7
Squamous cell carcinoma of the lung	1
Adenocarcinoma of the lung	5
Metastatic adenocarcinoma of the pleura	3, 4
Squamous cell carcinoma of the tongue	1, 4, 7
Meningioma	1
Endometrial endometrioid carcinoma	2
Endometrial seropapillary carcinoma	1
Thyroid carcinomas	1,4 , 7

Table 2. Cancers that display loss of claudins expression.

Malignancy	Claudins that show absent or weak expression
Breast carcinoma	1, 7
Ovarian sex cord stromal tumors	1,3, 4, 5
Gastric adenocarcinoma, poorly differentiated or diffuse type	4
Colorectal carcinoma	8
Hepatocellular carcinoma	4
Hepatoblastoma	3, 4, 7
Oncocytoma	7
Prostatic carcinoma	2, 5
Squamous cell carcinoma of the lung	5
Adenocarcinoma of the lung	1
Mesothelioma	4, 5
Head and neck squamous cell carcinoma	7
Endometrial endometrioid carcinoma	1
Endomtrial seropapillary carcinoma	2
Undifferentiated thyroid carcinoma	1
Metastatic melanoma	1

mRNA were greatly reduced in adenocarcinoma of diffuse type compared to the intestinal type (Lee et al., 2005). Loss of claudin 4 immunostaining was also lost in poorly-differentiated carcinomas, diffuse carcinomas and in diffuse areas of mixed histology carcinomas. Thus, expression of claudins 3 and 4 can be used as an adjunct diagnostic as well as prognostic tool to assess patients with gastric adenocarcinoma.

Colorectal carcinoma

Recently it was found that claudins 1 and 12 were over-expressed in colorectal carcinomas, while claudin 8 was down-regulated at the mRNA levels. By IHC, claudin 1 exhibited cytoplasmic enhancement of nonjunctional staining in primary colonic carcinoma samples, and cytoplasmic and nuclear staining in metastatic colonic carcinomas (Miwa et al., 2000). Thus, increased claudin 1 expression was associated with metastases in colon carcinoma, where metastatic cells showed highest expression of claudin 1 levels (Miwa et al., 2000; Dhawan et al., 2005). Colonic carcinoma cells, whose claudin 1 levels were suppressed by genetic manipulation using short interfering RNA (siRNA) molecules, were less invasive in the matrigel cell invasion assay and had less ability to migrate in the wound-healing migration assay (Dhawan et al., 2005).

Hepatocellular and biliary tract carcinomas

Overexpression of claudin 7 was reported in hepatocellular carcinomas (HCC) in a size-dependent fashion where along with villin, cell death factor CIDE-A and junB, it was specifically expressed in small tumors (<2 mm) of EGF-induced HCC. When the authors of this study compared genes expression profiles of HCC tumors of different sizes, claudin 7 expression was absent in both medium-size tumors (5 mm) and large size tumors (10 mm and above) (Borlak et al., 2005). Claudin 10 was also expressed in HCC and was associated with recurrence (Cheung et al., 2005).

Lódi et al, (2006) studied the expression of claudin 4 in biliary tract carcinoma using IHC, western blot analysis and RT-PCR and compared it to its expression in hepatocellular carcinoma. Biliary tract carcinomas exhibited intense membranous staining while normal biliary epithelium showed weak positivity for claudin 4. By contrast, normal hepatocytes and HCC tumors did not express claudin 4. Results of Western immunoblot analysis and RT-PCR were in correlation with immunohistochemical findings. Thus detection of claudin 4 seems to help in differentiating biliary tract carcinomas from HCC and could possibly become a potential diagnostic tool. Other studies have shown that claudin 4 was strongly present in extrahepatic bile duct cancers and gallbladder carcinomas whereas claudin 1 and 10 in intrahepatic bile duct carcinomas (Németh et al., 2008).

Pancreatic cancer

Claudin 4 is over-expressed in the majority of pancreatic carcinomas (Michl et al., 2003; Nichols et al., 2004). *In-vitro* studies showed that over-expression of claudin-4 was associated with decreased invasiveness. TGF-ß was identified as a negative modulator of claudin 4. TGF-ß is known for its late promoting effects of enhancing invasiveness and angiogenesis of tumor cells (Michl et al., 2003). Both exogenous and endogenous over-expression of TGF-ß resulted in decreased expression of claudin 4. By IHC 92% of pancreatic ductal carcinoma expressed claudin 4 and 58% claudin 1. Claudin 1 expression had an inverse correlation with histologic tumor grade, was more prevalent in hyperplastic foci and adenoma and was least expressed in invasive carcinoma (Tsukahara et al., 2005). In intraductal papillary mucinous tumor of the pancreas, claudins 1 and 4 showed distinct pattern of expression in various subtypes of this entity (Tsukahara et al., 2005).

Urinary tumors

Expression of claudins 1, 3, 4, 7 and 8 was identified in variety of renal cell tumors. Claudin 1 was detected by IHC in both non-neoplastic renal tissue and tumors. Weak expression of claudin 3 was present in Fuhrman's grades 1 and 2 clear cell carcinoma and upregulation of claudin 3 occurred with higher grades of renal cell carcinomas. A significant inverse relationship exists between claudin 3 and 4 expression and overall patient's survival in clear cell carcinomas (Lechpammer et al., 2008). Immunostaining for claudin 7 was significantly more common in chromophobe renal cell carcinoma while claudin 8 staining was more common in oncocytoma. The staining patterns of these claudins may also differ in various renal tumors from cytoplasmic to membranous. Other tumors stained to a less frequent rate. The differential expression of claudins 7 and 8 has been used successfully to differentiate between chromophobe renal cell carcinoma and oncocytomas (Hornsby et al., 2007; Lechpammer et al., 2008; Li et al., 2008; Oskunkoya et al., 2008).

Urothelial carcinomas also expressed claudins 1, 3, 4 and 7 in >80% of cases. Expression of claudins was found to be associated with higher stage and tumor grade (Nakanishi et al., 2008).

Prostate cancer

Claudin 3 mRNA was expressed in glandular epithelial cells in normal prostate tissue and in prostatic adenocarcinoma. In all cases where prostatic adenocarcinomas or intraepithelial neoplasias were present, mRNA levels of claudin 3, were equal to expression levels in surrounding normal epithelial cells within the same tissue section. IHC also confirmed strong expression of claudins 1, 4 and 7 (Long et al., 2001). In contrast, claudins 2 and 5 had weaker staining

than normal prostate tissue. Claudins 1 and 5 were more expressed in tumors with low Gleason score (Väre et al., 2008). Decreased claudin 1 expression was also found to correlate with high tumor grade and recurrence. Similarly, decreased claudin 7 expression correlated with high tumor grade as well (Sheehan et al., 2007).

Lung cancer

Striking differences have been identified in regards to the types of claudins and their expression in various lung cancers (Moldvay et al., 2007). The expression patterns of claudins 1 and 5 were found helpful in the differentiation between squamous cell carcinoma and adenocarcinoma. By IHC, squamous cell carcinomas were positive for claudin 1 and negative for claudin 5, while adenocarcinomas were positive for claudin 5 and negative for claudin 1 (Paschoud et al., 2007). Claudins have also reported to be helpful in differential diagnosis between mesothelioma and metastatic adenocarcinoma of the pleura. Claudins 1, 3, 4 and 7 were significantly less positive in mesothelioma than in metastatic adenocarcinoma and their expression was inversely associated with calretinin positivity. This was most evident in claudins 3 and 4, which were nearly as good as calretinin in mesothelioma detection. Claudins 3 and 4 were expressed in 18 and 23 % of mesotheliomas, and in 90 and 100% of metastatic adenocarcinoma of the pleura, respectively (Soini et al., 2006b).

Head and neck cancers

Al Moustafa and co-workers reported under-regulation of claudin 7 in a microarray study of head and neck squamous cell carcinomas (HNSCC). The results of the microarray cDNA analysis were validated using western blot analysis and QRT-PCR techniques on a three normal epithelial cell lines matched with HNSCC tumor cell lines (Al Moustafa et al., 2002).

In contrast, squamous cell carcinoma of the tongue showed strong expression of claudins 1 and 7, moderate expression of claudin 4 and minimal expression of claudin 5. Strong and low immunoreactivity of claudin 7 was associated with decreased survival compared with medium immunoreactivity (Bello et al., 2008). Oral squamous cell carcinoma cell lines with higher expression of claudin-1 by IHC and western blot exhibited a more aggressive invasive potential and stronger expression of the active form of the matrix metalloproteinase proteins compared to cells with lower expression of claudin 1 (Oku et al., 2006). Incubating cells with siRNA sequences directed at claudin 1 translated region resulted in significant reduction in the invasive potential of cell lines and reduction in the levels of matrix metalloproteinases (Oku et al., 2006).

Brain tumors

Claudin 1 expression has been found to be helpful in distinguishing meningiomas from other tumors arising from the meninges including solitary fibrous tumors of the meninges, meningeal hemangiopericytomas, and vestibular schwannomas. 53% of meningiomas were immunoreactive for claudin 1, whereas none of the other tumors were positive. Thus it appears that claudin 1 is specific marker for meningioma, however its sensitivity is low and it is better to use in a panel that includes other antibodies. Claudins were not expressed in other brain tumors such as glioblastoma multiforme (Swisshelm et al., 2005; Hahn et al., 2006).

Pediatric cancers and sarcomas

Claudins 1 and 2 were expressed in the fetal type of hepatoblastoma and were not expressed in the embryonal type. Claudins 3, 4 and 7 were also not expressed in any of hepatoblastoma histologic types (Halász et al., 2006).

Pediatric sarcomas with epithelial differentiation expressed one form or the other of claudins. Claudins 4, 7 and 10 were found expressed in biphasic synovial sarcomas, most closely associated with the epithelial component (Billings et al., 2004; Kohno et al., 2006). Claudin 1 expression was also identified in 63% of Ewing's sarcoma family tumors, consistent with its partial epithelial differentiation (Schuetz et al., 2005).

Other malignancies

Claudin 1 was expressed in benign nevi, dysplastic nevi and malignant melanoma in high percentage of cases. A correlation was identified between claudin 1 expression in primary melanomas and depth of Clark level/Breslow invasion. In contrast, metastatic melanoma tumors lose claudin 1 expression which was seen in only 19% of cases. Thus, loss of claudin 1 expression is associated with metastasis in melanomas (Cohn et al., 2005).

In endometrial tissues, intense protein expression was noted for claudins 3, 4, 5, and 7, without significantly different patterns in carcinoma, hyperplasia, secretory, and proliferative endometrium. Expression of claudins 3 and 4 at the mRNA and protein levels was increased in atypical endometrial hyperplasia and endometrial carcinoma, compared to normal or hyperplastic endometrial tissue (Sobel et al., 2006; Pan et al., 2007). In endometrial carcinoma, overexpression of these claudins was found to correlate with the depth of myometrial invasion. In endometrioid carcinoma, low claudin 1 and high claudin 2 protein contents were detected, in contrast to seropapillary adenocarcinoma which showed high claudin 1 and low claudin 2 levels. The two types of endometrial adenocarcinomas were well distinguished by claudins 1 and 2 by IHC (Sobel et al., 2006; Pan et al., 2007).

A differential expression of claudins in the different histologic types of thyroid gland tumors is also noted. Claudins 1, 4 and 7 were also expressed in various types

of benign and malignant thyroid neoplasms. Claudins exhibited reduced expression in undifferentiated carcinomas. Dedifferentiation of the thyroid carcinomas was accompanied by reduction in claudin 1, 4 and 7 expression. A correlation between loss of claudin-1 expression and worse disease-free survival was noted on univariate analysis (Tzelepe et al., 2008).

Conclusions

Claudins show variable expression patterns in different types of epithelial malignancies. This fact provides a platform for anti-cancer therapeutic research trials that target claudins molecules or TJs in general. However the ubiquitous presence of claudins in normal and hyperplastic tissues in addition to neoplastic tissues may limit the usefulness of any future anti-claudin therapy. Immunohistochemical detection of some claudins has also proved useful as a diagnostic tool that can differentiate between various types of malignancies. Certain claudins can also be used as markers that can predict patient's prognosis. Loss of claudins expression is also noted in several cancers and is related to metastasis in some cases. Thus it seems that identifying expression of claudins in various cancers is becoming increasingly useful in confirming the diagnosis, excluding other entities and judging patient's prognosis. Immunohistochemical detection of claudins will soon become part of the routine pathologic work-up of patients with various malignancies.

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