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Deep Insight Section

The Claudins family: Structure and function in normal and pathologic conditions

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Definition and introduction

Tight junctions (TJs) are the structures responsible for forming the seal that controls paracellular transport.

TJs are composed of multiple components, including the Occludin proteins, the Zona Occludin proteins and the claudin proteins.

The tertraspan integral membrane proteins known as claudins are essential for TJ formation and function (Tsukita and Furuse, 2000).

Twenty three claudin genes are found in the human genome. The exact mechanisms of claudin evolution remain unknown, although some data suggest that the claudin multigene family evolved through gene duplications early in chordate development (Kollmar et al., 2001).

In general, claudin genes are small, with few introns, or none at all (Lal-Nag and Morin, 2009). There is high degree of genetic homology among claudin genes, with several pairs showing similarity to each other in sequence and in intron/exon arrangement.

Many claudin genes are closely located in the human genome, such as claudin 6 and claudin 9 on chromosome 16, claudin 22 and claudin 24 on chromosome 4, claudin 8 and claudin 17 on chromosome 21 and claudin 3 and claudin 4 on chromosome 7 (table 1).

There is evidence that this genomic arrangement may result in coordinated expression as evidenced in co-expression of claudins 3 and 4 which has been reported in several normal and neoplastic tissues (Lal-Nag and Morin, 2009).

Translation of the aforementioned genes results in 23 distinct human claudin proteins.

These claudin proteins span the cellular membrane bilayer four times, where the N- and C-termini are oriented towards the cytoplasm and there are two extracellular loop domains (Morita et al., 1999). The C-Terminal PDZ binding motifs in each claudin binds other tight junction cytoplasmic proteins such as ZO-1, ZO-2, and ZO-3, MUPP-1, PALS-1 associated TJ protein (PATJ).

Stabilization of a tight junction is specifically dependent on interaction of claudins with cytoplasmic scaffolding proteins ZO-1 and ZO-2 which link claudins to actin cytoskeleton (Umeda et al., 2006).

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 can also change in the same epithelium according to physiological and pathological conditions, in response to pharmacological changes and growth factors and hormonal stimulation (Balda et al., 1991).

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).

Claudin member	Gene location (chromosomal regions)	Protein size (amino acids)	Protein size (Daltons)
1	3q28	221	22744
2	Xq22.3	230	24549
3	Xq11.23	220	23319
4	7q11	209	22077
5	22q11.21	218	23147
6	16p13.3	220	23292
7	17p13.3	221	22418
8	21q22.11	225	24845
9	16p13.3	217	22848

Table 1: Comparison of common claudins' gene locations and protein sizes.

Role of claudins in tumorigenesis, tumor progression and metastases

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; Ouban and Ahmed, 2010; Ouban et al., 2012).

While the exact functions of claudins in cancer cells are not fully understood, recent work strongly suggests that claudins are involved in survival and invasion of tumor cells (Agarwal et al., 2005; Morin, 2005; Dhawan et al., 2005; Kominsky, 2006; Oku et al., 2006; Dos Reis et al., 2008).

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 downregulation of claudins, because tumorigenesis is accompanied by disruption of tight junctions, with resultant loss of cohesion, invasiveness and lack of normal process of differentiation. However, it is interesting to note that numerous other studies have shown upregulation of these proteins in other cancers including, for 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), claudin 1 in oral squamous cell carcinomas (Dos Reis et al., 2008; Oku et al., 2006; Ouban et al., 2011). 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). It is known that in addition to their function as a seal controlling the paracellular transport, tight junctions also play critical roles in maintaining cell polarity and signal transductions (Tsukita and Furuse, 2000; Van Itallie et al., 2006).

It is through these functions that an over-expressed claudin protein may get involved in carcinogenesis and/or metastases. For example, claudins are important regulators of signal transduction from the cell-cell contact region (Gonzalez-Mariscal and Nava, 2005) and have also been shown to be directly recruiting and enhancing the activation of pro-matrix metalloproteinase 2 (MMP2) (Miyamori et al., 2001). MMP2 over-expression suggests a potential risk for invasion and metastasis in high-grade squamous intraepithelial lesions (Nasr et al., 2005) and pro-MMP2 activation is involved in pancreatic cancer progression (Ellenrieder et al., 2000).

CLDN1 has also been shown to be involved in the beta-catenin-Tcf/LEF signaling pathway and its over-expression was suggested to have a role in colorectal carcinogenesis (Miwa et al., 2000). The epidermal growth factor receptor (EGFR), has been suggested to regulate claudin proteins. EGF-induced EGFR activation increased CLDN1 expression in Madin-Darby canine kidney cells (Singh and Harris, 2004). EGFR is frequently amplified and over-expressed in many cancers, such as brain tumors (Schwechheimer et al., 1995), hepatocellular carcinomas (Tang et al., 1998), and head and neck carcinoma (Xia et al., 1999; Garnis et al., 2004); and increased expression of EGFR protein has been associated with worse prognosis (Bankfalvi et al., 2002).

The function of claudins in cancer is complex and diverse, with both over- and under-expression being linked to tumorigenesis. While the exact mechanism through which a claudin protein may predispose to carcinogenesis and metastases is still not clear in all cases, it is important to note the involvement of claudins in activation/recruitement of collagenases, activation of molecular neoplastic pathways such as the Wnt/Beta-catenin-TCF/LEF pathways, or with a growth factor well-known for its involvement in many tumor formations (EGF).

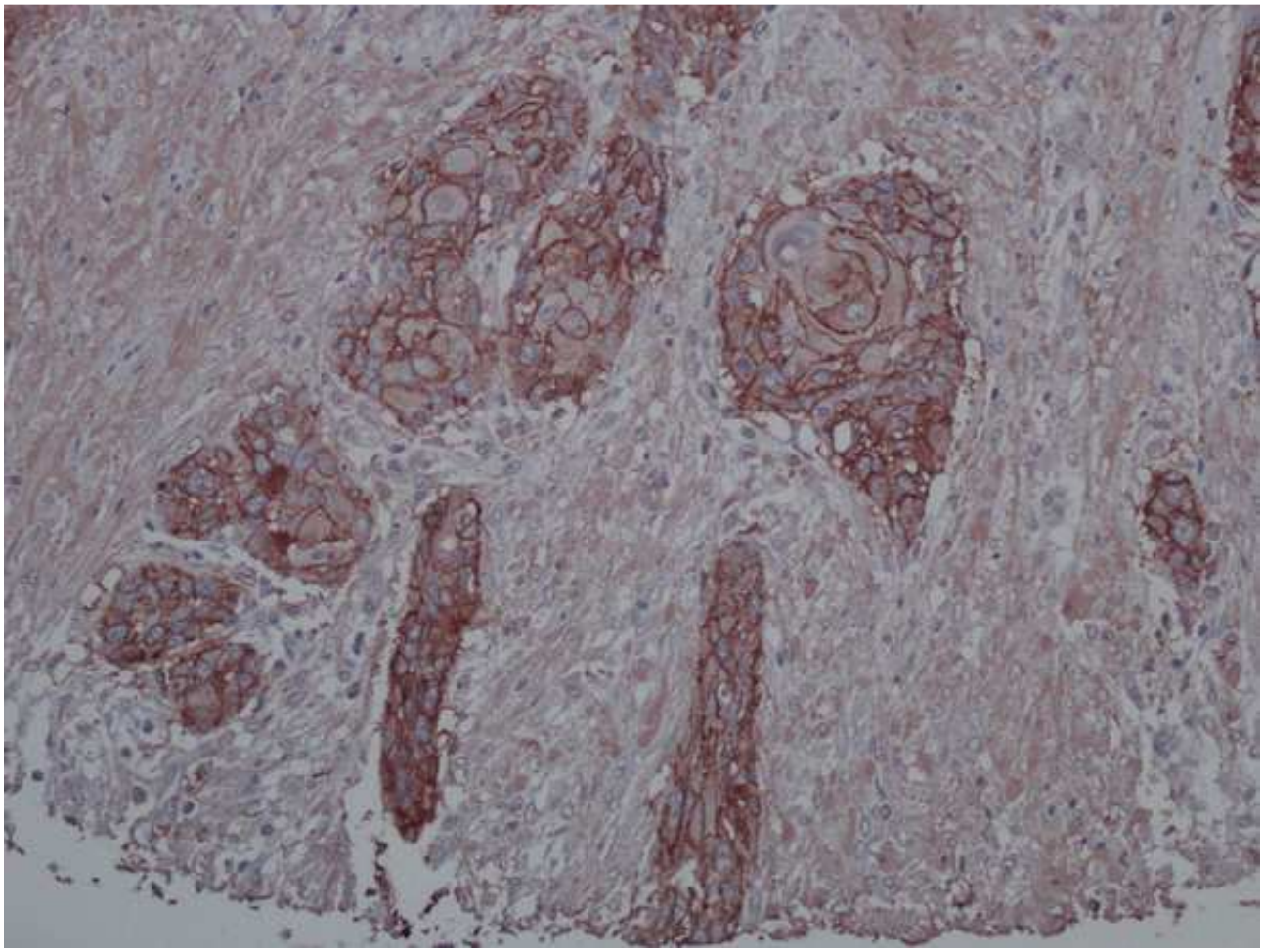

Figure 1. Immunohistochemical detection of claudin-1 in squamous cell carcinoma of the esophagus.

Furthermore, the deregulated claudin protein may in fact result in further weakness of the tight junction of epithelial cells, resulting in porous gaps with influx of growth factors, hormones and toxins through paracellular spaces providing a habitable environment for tumor cells (Amasheh et al., 2002).

And while much work is in progress on this matter, it is important to note that claudin proteins expression may have significant clinical relevance (Morin, 2005; Swisshelm et al., 2005). For example claudin 10 expression in hepatocellular carcinoma (Cheung et al., 2005), claudin 1 expression in colorectal carcinoma (Dhawan et al., 2005), and claudin 1 expression in oral squamous cell carcinoma (Oku et al., 2006; Dos Reis et al., 2008) have all shown values in predicting behavior of tumor and prognosis for the patient.

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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