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Review

Claudins and cancer: Fall of the soldiers entrusted to protect the gate and keep the barrier intact

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ABSTRACT

The role of the tight junctions (TJ) in controlling paracellular traffic of ions and molecules, through the regulation of claudin proteins, is now established. However, it has also become increasingly evident that claudin proteins, as integral components of the TJs, play crucial role in maintaining the cell–cell integrity. In conformity, deregulation of claudin expression and cellular distribution in cancer tissues has been widely documented and correlated with cancer progression and metastasis. However, this correlation is not unidirectional and rather suggests tissue specific regulations. Irrespective, if the widely described correlations between altered claudin expression and cancer initiation/progression could be established, they may serve as important markers for prognostic purposes and potential therapeutic targets. In this review, we summarize data from screening of the cancer tissues, manipulation of claudin expression in cells and animals subjected to cancer models, and how claudins are regulated in cancer. The focus of this article remains analysis of the association between cancer and the claudins and to decipher clinical relevance.

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Contents

1. Introduction to claudins

The majority of the cancer-associated deaths (~95% cases) represent cancers originating from an epithelium, and include cancer originating from the oral cavity, esophagus, stomach, colon, rectum, prostate, ovary, bladder, kidney, lung, pancreas, breast and liver [1–5]. Notably, despite the variable morphology and the specialized function of the differentiated epithelium in different tissues and organ systems, epithelial cells share few common traits including the polarized architecture. Incidentally, a common determinant that characterizes transformed epithelium in different cancer types is the loss of the polarized architecture. This is why there has been a constant craving among the scientific community to understand the cellular processes that either mediate the transformation of normal epithelial cells to acquire tumorigenic phenotype, or are modulated in this process, as intersecting these deregulations may hold the therapeutic promise to cure cancer. Like any physical entity with defined perimeters, a polarized epithelial cell

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requires the cytoskeletal meshwork and cell–cell adhesion proteins between the neighboring cells to provide the physical strength to keep their polarized architecture intact. In this regard, a critical requirement of the tight junctions (TJs), the most apical cell–cell adhesions, is widely recognized as the keeper of the "fence function", possibly due to their strategic location, and also because transformed cells do not possess normal and functional tight junctions [6,7]. Furthermore, the transformed epithelium is characterized by leakiness, which again indicates toward deregulation of the "gate (or barrier)" function of the tight junctions. However, despite the evidence supporting a causal association between the deregulation of TJ-structure/function and cancer, advances in this area were limited until 1998 when Tsukita and his group reported that the claudin family of transmembrane proteins are integral to the structure and function of the TJs [8,9]. However, since their discovery, investigations aimed at understanding the role of these proteins in normal and pathological cellular processes including cancer have progressed at a rapid rate. These studies have not only clarified the previously unclear molecular details of the structure and normal functioning of the tight junction but also unraveled role of these proteins in functions other than the conventional tight junction function including differentiation, stem cell niche, migration and invasion to name a few.

The name "claudin" comes from the Latin word "claudere" ("to close"), which fits the barrier role of these proteins. The claudins are transmembrane proteins and currently there are 27 known claudin members in this family, though claudin-13 is missing in humans [7,10–12]. Notably, based on their sequence similarity, which is determined by the alignment and phylogenetic tree analysis of the whole-length sequences, two subgroups of claudin family members have been recognized: the classic and the non-classic claudins [7,13]. The classic claudins exhibit a much stronger sequence homology and include claudins 1–10, 14, 15, 17, and 19, and the non-classic claudins contain claudins 11–13, 16, 18, and 20–24. Although research investigating claudin biology has largely centered on classic claudins, possibly due to the availability of the antigen-specific antibody, no functional diversity has yet been identified between the classic and non-classic claudins. Most claudin proteins are within the 20–34 kDa size range and are reported to have four transmembrane helices with amino- and carboxyl-terminal tails extending into the cytoplasm [7,14,15]. Structurally, the first extracellular loop, among two extracellular loops in a claudin protein, contains charged amino acids to regulate paracellular ion selectivity of anions and cations [15]. However, first extracellular loop of claudin-2 protein is also essential for mediating breast tumor cell–hepatocyte interaction and the ability of breast cancer cells to form liver metastases *in vivo* [16]. The carboxy-terminal tail of claudins, which mostly differ in size and sequence between different claudin proteins, contain a PDZ-domain-binding motif that allows claudins to interact directly with cytoplasmic TJ-associated proteins including ZO-proteins. Moreover, this tail region is also the site of post-translational modifications such as phosphorylation, which can affect the localization and functions of claudins [17,18]. These deregulation of claudin proteins and interaction with other proteins have been of particular interest to the cancer biologists. The expression pattern of claudins varies among tissue types, which is why use of claudins as cancer biomarkers is highly desired. Furthermore, most tissues or cell types express multiple claudins and multiple combinations of claudin expression can contribute to the formation of TJs through their homotypic or heterotypic interactions, or their interaction with other TJ proteins [9]. The possibility that claudins may participate in multi-protein complexes to regulate cell behavior gets support from recent finding that pull-down of claudin-2, a ~22 kDa protein modulated in many cancers, precipitates ~720 kDa protein complex [19].

2. Epithelial to mesenchymal transition (EMT), claudins and cancer progression

The primary cause of the cancer-associated deaths is malignancy due to the metastasis of cancer cells from primary tumor site to distant vital organs. The key event that is believed to empower the cancer cells to metastasize is the epithelial to mesenchymal transition (EMT). During this process, epithelial cells downregulate cell–cell adhesion structures, alter their polarity, reorganize their cytoskeleton, and become isolated and motile. Thus, downregulation of several claudins in cancer is consistent with the disruption of tight junctions during tumorigenesis and EMT [20,21]. Furthermore, forced induction of EMT in epithelial cells results in significant loss of claudin expression and TJ-function. However, changes in claudins in cancer-related EMT present a more complex situation than E-cadherin, the adherens junction protein, which is suppressed in epithelial cells undergoing EMT in a ubiquitous manner, as not all claudin family proteins are downregulated in cancer cells. In fact, specific claudin family members especially claudin-3 and -4 have rather been documented to be upregulated in multiple cancer types including prostate, pancreatic and ovarian cancer (Fig. 1) [22–24]. Importantly, sole reliance upon the total cellular levels of claudin expression in cancer cells may elude us from true understanding of the role claudin proteins in cancer regulation, as sheer deregulation of these proteins from their membrane localization (without a decrease in transcription and/or protein synthesis) can modulate the cellular homeostasis in a significant manner. In this regard, we and others have demonstrated an increased however mislocalized (cytosolic/nuclear) expression of claudin-1 in colorectal cancer [25].

Similarly, nuclear localization of claudin-2 in correlation with the cellular proliferation has been demonstrated [26]. Recent studies further suggest that claudin expression may even be distinct within specific subtypes of the cancers: for example, claudin-4 expression is downregulated in grade 1 ductal carcinoma of the breast compared to normal mammary epithelial cells [27], whereas it is significantly upregulated in basal-like breast cancer [28]. Similarly, expression of claudin-1, -3 and -4 is higher in the intestinal type of gastric adenocarcinoma than in diffuse type of gastric cancer [29]. A recent comprehensive analysis of the expression of claudins-1, -3, -4, -7 and -8 in high-grade invasive breast cancer, including several molecular subtypes, further demonstrated differential expression of claudins according to the molecular subtype, showing increased claudin-7 and -8 in luminal tumors (estrogen positive) and increased claudin-1 and -4 in basal-like tumors [30,31]. Accordingly, a new claudin-low molecular subtype of breast cancer is now recognized, which is characterized by low expression of the tight junction and adherens proteins, including claudin-3, -4 and -7, and E-cadherin [32], and enriched in stem-like and EMT features [33,34]. These observations implicate a potential role for the tissue microenvironment in diverse and often contrasting regulation of claudin proteins in different cancer types and further emphasize their potential as the biomarkers. Notably, we and others have demonstrated contrasting EGF-receptor-dependent regulation of claudin-2 expression in renal *versus* lung and colonic epithelial cells, possibly through the influence of the microenvironment (fibroblast–epithelial interaction) [35–37]. Overall, the diverse and tissue-specific changes in claudins expression highlight potential of the altered protein partnering in regulating tissue-specific events promoting carcinogenesis, and suggest role of the tissue microenvironment and dependence upon