Claudins, Inflammation and Epithelial-Mesenchymal Transition in Gastric Tissue

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Abstract

Gastric cancer is a serious worldwide health burden. It is the fourth most frequently diagnosed cancer with an estimated 1 million new cases per year. The disease is often diagnosed in advanced stages and is associated with a poor prognosis for patients. It exhibits heterogeneity in clinical, biologic, and genetic aspects. Although H. pylori is the best studied risk factor, interleukins associated with chronic inflammation, disruption of tight junctions especially claudins, MERK/ERK signaling pathways and cancer stem cells have key roles in carcinogenesis and progression. An in-depth understanding of the role that inflammation and modified tight junction proteins play in epithelial-mesenchymal transition may improve our understanding of the cancer process and lead to the recognition of new biomarkers for early diagnosis, and possibly, improved therapeutics.

Keywords: Chronic inflammation; Epithelial-mesenchymal transition; Gastric tissue

Introduction

The mammalian gastric mucosa and its glands are formed by simple cylindrical mucus secreting epithelium, and gastric tubular glands reaching the muscular layer, underneath; the structure of these glands differs according to the gastric anatomical area [cardias, body, pylori] [1]. Epithelial cells are attached to each other at their lateral membranes by a complex of intercellular junctions that maintain the cells together. The most apical of them is the occluding junction also known as zonula occludens, or tight junction. This complex is composed of multiple proteins that include transmembrane proteins, cytoplasmic signaling proteins, and adapters that link them to the actin cytoskeleton [2]. The transmembrane proteins mediate the major functions of the tight junction: barrier, pore, and fence. There are single transmembrane domain proteins [JAM, Crb3, CAR], a triple transmembrane domain protein [Bres], and the four-transmembrane domain proteins, the claudin family and TAMP [occludin, tricellulin, Marvedl] [3].

Claudins are barrier-forming proteins. They regulate paracellular permeability, can form pores, especially small pores [-4 Å], or enhance water permeability. They are considered the major determinants of the permeability properties of epithelial cells. There are 27 claudins in mammals grouped in eight subgroups [3,4]; they are expressed in a tissue-specific pattern and distributed in all the cell-cell contact areas in epithelia. There is abundant evidence on the function and tissue specificity of claudins [2,5]. Multiple claudin isoforms are expressed simultaneously at the tight junction. Using the serial analysis of gene expression [SAGE] Genie database it has been confirmed that some tissues express a large number of claudins genes whereas others show a more restricted pattern [6]. The arrangement of claudins give raise to two extracellular loops, the first play crucial roles in paracellular charge selectivity whereas the second is important in tight junction strand interactions between claudins located in neighbor cells (Figure 1). Despite the physiological functions of the first and second extracellular loops of claudins, the long and highly divergent C-terminal tail of claudins are related to various important functions. Firstly, the C-terminal is essential for trafficking to the tight junction [7], since its truncation lead to claudin intracellular retention and subsequent degradation; this tail is also vital to determine the protein half-life [8] but it has also been recognized as a regulator of some homeobox genes [9] related to embryo development [10]. Claudin expression can be regulated at the transcriptional, posttranscriptional, and posttranslational levels. The regulators of claudin expression comprise some homeobox transcription factors such as CDX1, CDX2 and HNF-1a as well as TNF-a/NFkB and TGF-b-Smad/Snail pathways, PPARy, SP1, HNF-4a, GATA-4 and Grhl2.

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Claudin Expression in Gastric Tissue

As already mentioned, each claudin isoform is expressed in a tissue-specific pattern. In rodent stomachs, claudin-3 is most strongly expressed in the surface epithelial cells along the basolateral membrane, claudin-4 is expressed mainly at the tight junction in proximal gastric glands, and claudin-5 is uniformly expressed from the base of the glands to the surface on the basolateral membrane [11]. Claudin-21, -24 and -25 are expressed in the stomach between day 7 and 17 of mouse embryo development [3].

Developmental changes in claudin expression during human neonatal life have been observed in the jejunum and intestine [12,13]. Claudin-2 epithelial expression at birth decreases markedly over 90 days whereas claudin-19 is no longer expressed 28 days after birth. Claudins-3, -4, and -7 increase its expression in mesodermal-derived tissues during early life, and claudin-15 migrates from the crypts to the jejunal surface epithelium [2]. Despite all this, there are reports of nuclear localization of claudins, suggesting a direct role in the regulation of gene expression; however their physiological function remains to be determined.

The mammalian stomach is a histologically complex organ protected with a simple cylindrical epithelium, the mucosa and the so-called gastric fossae. The fossae gastric glands are composed by neck, principal, parietal, enteroendocrine, and non-differentiated stem cells are found. The composition of claudins plays an essential role in the physiological function of the gastric TJ. Claudin-6 is one of the earliest molecules expressed during epithelial differentiation, and its expression is largely confined to embryonic and fetal life [14-16]. Nevertheless, the expression of claudins in human stomach development is not known. Claudin-6 has been detected in human fetal cells isolated from maternal blood [17]. Claudin-18.2 expression appears to be exclusive and restricted to exocrine and endocrine cells of the gastric glands [18]. Our initial results in human fetal gastric samples show that claudin-6 and claudin-9 are temporally expressed between the 3- and 7-week [data not shown]. Claudins-12, -18.2, and -23 and, are highly expressed in healthy adult human stomach [19]. As claudin expression is tissue specific, a definite knowledge of the claudins normally expressed in the different regions of the stomach, is vital.

Claudins and Epithelial-mesenchymal Transition

Epithelial-mesenchymal transition [EMT] is a developmental process whose essential features are the disruption of intercellular contacts and the enhancement of epithelial cell motility, migratory properties of mesenchymal cells and the acquisition by the latter of stem-cell like properties [20-22]. This transition is defined by the progressive loss of epithelial protein markers [E-cadherin, Cytokeratin, Laminin-1, Entactin, Syndecan, MUC1, Desmoplakin, a1IV collagen, miR200 family and ZO-1] and gain of mesenchymal markers [N-cadherin, Vimentin, Fibronectin, FTS binding protein FAP, Syndecan-1, a5b1 integrin, miR10b, miR21, FOXC2, LEF-1, ETS, and SFP1]. In this transition transcription factors such as Snail, Slug and Twist play a decisive role. There are different EMT types. Type 1 is associated with implantation and embryonic gastrulation, type 2 is associated with wound healing, tissue regeneration and organ fibrosis, and type 3 occurs in the neoplastic transformation process [23]. Inflammatory cytokines are associated with EMT and carcinogenesis. Nevertheless, the modifications in claudin expression must be subordinated to major modification in the signaling pathway. In EMT intercellular adhesion decrease, cell motility increases, synthesis of extracellular matrix proteins increases, there is a loss of cell polarity and elevated resistance to apoptosis, and all these phenomena require dynamic changes of TJ protein expression. The latter depends, amongst many others, on the phosphorylation level of the claudins [24-26]. MAPK activation inhibits the formation of TJ whereas inhibition of MEK1 signaling permits TJ formation [27]. TJ formation is also induced by the complex formed by the atypical protein kinase C [aPKC], PAR3, and PAR6 [28]. Since claudin determine the ion selectivity of pores, is it essential or necessary for EMT a modification in ion selectivity?

HGF, EGF, PDGF, and TGF-β, appear to be responsible for the induction or functional activation of a series of EMT-inducing transcription factors, notably Snail, Slug, zinc finger E-box binding homeobox 1 [ZEB1], Twist, Goosecoid, and FOXC2 [29,30]. Once expressed and activated, intracellular signaling networks involving, ERK, MAPK, PI3K, Akt, Smads, RhoB, β-catenin, lymphoid enhancer binding factor [LEF], Ras, and c-Fos as well as cell surface proteins such as p4 integrins, α5b1 integrin, and αvβ6 integrin [31] facilitate the transition. Activation of EMT programs is also facilitated by the disruption of cell-cell adherens junctions [23,32]. Therefore the modification of intercellular ion exchange becomes necessary since the cells generated in this epithelial-mesenchymal transition possess stem cell properties. Embryonic stem cells differentially express claudin-4 during early stages of hematopoietic commitment [33]. Claudin-4 is considered as a real marker of EMT because it increases the number of TJ strands and trans-epithelial resistance but at the same time it decreases the permeability of Na+. Claudin phosphorylation associated with tight junction disassembly is also enhanced by EphA1, which is recruited to bind to claudin-4 by forming a complex with epithrin-B1 [34]. Interestingly ephrins induce EMT [35,36]. F9 cells, an embryonic carcinoma cell line, forms di novo functional tight junctions expressing claudin-6 and claudin-7 under HNF4alpha induction [37,38]. Stem cells express claudin-6 because it is vital for their survival and self-renewal [39] and is considered a marker of cancers with a primitive phenotype [40].

The transcription factors beta-catenin/Tcf complex [41] and Cdx homeodomain protein/hepatocyte nuclear factor-1α bind directly to claudin promoters [42], thus regulating TcJ activity under physiological and pathological conditions. The transcription repressors E12/E47, ZEB-1, SIP-1, Snail, and Slug binds to E-box motifs in claudin-3, -4 and -7 promoters thus suppressing expression [43].

Up-regulation and modification of tissue claudins may also affect cell signaling pathways via binding domains to ZO-1 that interacts with signaling proteins AF-6, connexin 43, or G proteins [44-46] associated with neoplastic process.

When the epithelial cells are activated and the EMT process is initiated the simultaneous loss of epithelial characteristics and cell-cell adhesion is required so the modified cells gain mobility and ability to cross the basal membrane, therefore loss or modification of cell polarity is a priority [47]. This process possibly fulfills a more physiological role in the carcinogenesis environment. It has been shown that NFkB dependent increase of transcription factors related to the development of cancer stem cell properties, thwarts the irreversible loss of epithelial identity, thus maintaining the carcinogenesis process [48,49]. EMT is also related to repression of some kinases important in the regulation of the structure and function of the tight junction [50]. If the basic concept of epithelial carcinogenesis is that an epithelial cell is becoming de-differentiated, then the modified cell must express proteins compatible with its new differentiation and functional status, similar or identical to those determined during embryogenesis. Therefore tight junction proteins should contribute to maintain the epithelial integrity of cell.
layers in the context of a normal developing embryo in accordance to its differentiation markers [51].

Claudins in Cancer

The expression of claudin proteins is altered in neoplastic tissues and their role may be linked to functions unrelated to tight junction formation, essentially survival and invasion of cancer cells [52-54]. Nevertheless, the relationship between claudin expression and development of cancer requires a better understanding of the regulatory network [55]. Epithelial-to-mesenchymal transition (EMT) is central to cell migration and thus invasiveness and metastasis in cancer. Several pathways intertwine to regulate this transition and many of their transcription factors are involved in the regulation of claudins expression. The most important signaling pathways involved in EMT seem to be related to TGF-β, a cytokine that induces de-differentiation of cells [56], but many other transcription factors regulate the expression of claudins. For example, HNF-4α up regulate the expression of claudins-6 and -7 and induces epithelial differentiation through their translocation to the tight junction [37] in embryonal carcinoma cells [57] but at the same time it also inhibits the development of stable epithelial cell layer [58]. Increasing evidence emphasizes the role of up- or down-regulation of signaling pathways as well aberrant proteasomal activity in carcinogenesis [59,60]. Claudin-1, -3, -4 and -7 are amongst the most frequently deregulated claudins in various cancers but significant differences have been reported depending the stage and grade of the cancers samples. Claudin-6 is also detected although under-expressed in primary mammary gland tumors [61]. Claudin-7 is expressed in epithelial tissues [62]; acts as a paracellular Cl⁻ pore and form a complex with the epithelial cell adhesion molecule [63] thus regulating cell-cell adhesion, cell motility, and tumor progression. Claudin-7 is also under-expressed in primary breast as well as head and neck tumors [64]. Claudin-9 is mainly expressed in the cochlea [65]. Claudin-18-2 is a major constituent of tight junctions in stomach epithelia [66] and it induces a selective sealing of the tight junction against H⁺ [67] thus protecting the epithelium against low pH. Claudin-23 has also been detected in stomach and it appears to be deregulated in gastric cancer [68].

Claudins in Gastric Cancer

Of the great myriad of cancers affecting the population, gastric cancer is still the fourth most common cancer and the second most common cause of cancer death in the world. The incidence is higher among men and the prognosis is poor. Nearly 1 million new cases of gastric cancer are diagnosed worldwide annually. Risk factors include Helicobacter pylori infection, smoking, high intake of salt-preserved food, dietary nitrite, low intake of fruit and vegetables, family history of cancer, and gender [69]. Around 95% of gastric cancers are adenocarcinomas, which are classified into “intestinal” [92]. The MyD88 signaling of “danger signals” [PAMPs and DAMPs] by TLRs or NOD1/2. Inflammasome also sense PAMPs and DAMPs by NLRs [Nod-like receptors] or AIM2 [absent in melanoma 2]. Activation of NLRs result in the activation of pro-caspase-1, which once cleaved can catalyze the induction of epithelial to mesenchymal [EMT] transition [90]. EMT and Inflammation in Gastric Cancer

A major concern in relation to gastric carcinogenesis would be the function that inflammation might play in the process. Chronic inflammatory responses are associated with various types of cancer. To maintain homeostastic tissue function, innate immune system recognizes altered levels of self-molecules as well as foreign molecular structures. A simplified pathway would be the activation of a group of multimeric protein complexes called the inflammasome complex. Inflammasomes promote inflammation and inflammatory cell death through the activation of cysteine protease caspase-1. Two steps activate Inflammasomes. Initially, the expression of pro-IL-1β and pro-IL-18 [cytokines structurally related to IL-1β] is induced through the activation of NF-κB [85]. NF-κB can be activated by TNF-α and IL-1 or by sensing “danger signals” [PAMPs and DAMPs] by TLRs or NOD1/2. Inflammasome also sense PAMPs and DAMPs by NLRs [Nod-like receptors] or AIM2 [absent in melanoma 2]. Activation of NLRs result in the activation of pro-caspase-1, which once cleaved can catalyze the activation of IL-1β and IL-18. [86,87]. Cancers of the gastrointestinal tract are frequently associated with chronic inflammation, especially gastric cancer where the link between Helicobacter pylori infection and chronic gastritis is well documented. There is evidence linking inflammasome and their IL-1β and IL-18 products to gastric cancer [88]. In fact, IL-1β in stomach induces tumorigenesis [89] through the induction of epithelial to mesenchymal [EMT] transition [90]. EMT is a process involved in normal embryonic development and repair of epithelial injury but it is also implicated in carcinogenesis [91]. IL-1β and IL-18 utilizes MyD88 as an adaptor protein that stimulate the IL-1R and IL-18R signaling pathway that required for NF-κB and MAPK activation. The Toll/IL-1R domain of MyD88 interacts with the TIR domains of IL-1R1 and also with IRAK1 and IRAK2. The role of MyD88 signaling in the regulation of inflammation during cancer progression of the intestine [92] has been documented. The MyD88 signaling
pathway control inflammation in tissue repair [93,94] but upon injury the MyD88 signaling pathway is enforced. It has been suggested that tumor development is a continuous and unregulated state of tissue repair and stem cell renewal [95] secondary to abnormal homeostasis. An interesting question is whether the process derives from tissue stem cells or from cancer stem cells. Cancer stem cells might be derived from de-differentiated of progenitors that undergo genetic or epigenetic changes. Experiments in Drosophila testis have shown that germinal stem cells are highly dependent of the Jak-STAT signaling pathway [96]. Wingless [Wnt] signaling pathways, important in tissue regeneration and cancer growth [95], target claudin1 gene [97]. Wnt signaling and Cdx transcriptional activation regulate claudin-2 promoter activity [98]. Similarly, it has been shown that H pylori CagA+ strains increases intestinal cell proliferation by Wnt pathway activation [99] and inflammation stimulates epithelial cells to promote Wnt signaling activity [100]. The cancer stem cells possess features of the so-called EMT cells and through Snail-1 transcription factor, an inducer of EMT cells, these cells have highly activated NF-kB/MAPK signaling pathways in the presence of IL-1β and IL-8 [101] and have enhanced Wnt activity.

A serious concern is how does the inflammatory environment influences these signaling pathways and transcription factors related to modifications in claudin expression. In the majority of H. pylori-infected individuals an asymmetric chronic gastritis develops, the symptomatic disease occurs in approximately 10% of infected individuals. This difference may reside in H. pylory strain-specific virulence factors, genetic predisposition and/or a higher inflammatory response. Nevertheless, H. pylori and particularly CagA+ strains, is the only bacterium clearly associated with development of cancer [102]. The cytotoxin-associated gene A, activates ERK-MAPK pathway [103,104], induce the “hummingbird” phenotype [105], is associated with an increase in cell proliferation, EMT [106], inhibition of apoptotic signals [107], activation of the IL-6/gp130 receptor [108], activation of NF-kB and IL-8 [109,110], angiogenesis [111], regulation of stem cell differentiation [112], and disruption of the innate and Th1, Th17 and Treg balance [113].

Chronic H. pylori infection increases the epithelial expression of TLR-2, -4, -5 and 9 as well as IL-8, IL-10 and TNF-α, in gastric mucosa [114]. This chronic production of the inflammatory cytokines initiates a negative feedback of NF-kB, AP-1 and CREB-1 activation thus perpetuating the synthesis and secretion of the inflammatory cytokines. We have been evaluating the effect of H. pylori CagA+ and CagA+ strains on human gastric adenocarcinoma [AGS] cells ability to secrete inflammatory cytokines. The results show that CagA+ strains are powerful inducers of IL-1β and IL-8 secretion whereas CagA+ strains have a weak effect. Obviously, when various strains were evaluated there were no differences in the quantity of secreted cytokine but in the exposure time needed to exercise the inducer effect. We attribute the differences to the presence of other infectious toxins such asVacA in the H. pylori strains we evaluated. In co-activation experiments we also found that AGS cells grown in a cytokine milieu representative of a chronic inflammatory status secrete IL-8 in extremely high concentrations (>3,000 pg/ml). As a consequence of these results we evaluated the effect of these pro-inflammatory cytokines upon claudin expression in AGS cells. The results showed that the expression of claudins-5, -6 and -7 was up-regulated by this cytokines but most interestingly, we found that the expression of claudin-4 was strongly up-regulated when IL-8 concentrations above 2,500 pg/ml were used. It has been recently shown that in co-cultures of fibroblasts with malignant epithelial cells IL-8 is secreted to the culture medium and that this cytokine contributed to the maintenance of a low-differentiation phenotype of the epithelial cells [115]. A similar observation has been recently reported in the mammary gland epithelial cell line MCF10A [116].

**Clinical Implications of Claudins, EMT and Inflammation**

Claudin modification has been clearly established in several cancers including gastric cancer [117-119] and the loss of cell polarity, a function clearly regulated by tight junction proteins [120], is considered a hallmark of EMT. Interleukin 8 participation in oncogenesis has been clearly established [121], therefore in the frame of all these considerations it is clear that the sole prophylaxis of H. pylori infection with antibiotic therapy might not be sufficient to avoid gastric carcinogenesis [122] especially when pre-malignant lesions are already present. Serious efforts to develop an H. pylori vaccine are underway but information regarding possible host advantages in the host life is emerging [123,124].

Efforts to modulate the expression of claudin are underway. It has recently been shown that pegylated interferon and ribavirin modulate claudin and E-cadherin expression in HCV-infected cell lines [125], an infection strongly associated with cancer development. Similarly, Histone deacetylase inhibitors are being considered as promising anticancer drugs due to its effect on claudin-1 regulation [126]. Information regarding the inhibition of EMT in various cancers is emerging. Resveratrol, a compound found in grapes and red wine inhibits EMT in lung cancer [127] similarly to Celastrol, also known as tripterine, that also has inhibitory effects [128]. In the early stages of gastric neoplastic transformation the overexpression of gastrokine 1 might protect against MET [129]. The use of anti-HER2 drugs is promising but apparently only in HER2-positive gastric cancer [130].

The spectrum of possible therapeutic agents is promising, but our aim ought to be focused in determining cell markers that accurately point to the early changes associated with carcinogenesis. The presence of claudins associated with cell de-differentiation, or other markers associated to severe inflammatory processes or early EMT, such as claudins 4, 6, 7 or 9, CD44, Erb-2, E-cadherin or N-cadherin in the gastric biopsies should warn the clinician. We strongly believe that to routinely include the search of these markers will favor the early detection of cancer increasing survival rates and reducing medical costs.

**Conclusive Paragraph**

Gastric carcinogenesis is a multifactorial process. Genetic predisposition and risk factors have a triggering effect but currently one of the major associations is H. pylori CagA+ infection. The majority of well recognized gene associations are related to inflammation, matrix metalloproteinases, polymorphisms ofTLR’s or miRNA’s but the complexity involved in gastric carcinogenesis is reflected in the heterogeneity in clinical, biologic and genetic aspects. Among the great variety of processes in the progression of a normal epithelium to gastric cancer it appears that inflammation and modification of tight junction proteins, provoked by bacteria, diet, tobacco, drugs and others, induce signaling pathways that promote epithelial-mesenchymal transition. This review highlights the relation that apparently unrelated phenomena such as claudin modification – inflammation – and EMT, has in gastric carcinogenesis, and emphasizes the role that claudins might have in the early detection of gastric cancer. Nevertheless, a better understanding of these phenomena is clearly needed.
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References


Li Y, Wang L, Pappan L, Gallher-Buckley A, Shi J (2012) IL-1β promotes...
stemness and invasiveness of colon cancer cells through Zeb1 activation. Mol Cancer 11: 87.