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## Tight junction disruption: *Helicobacter pylori* and dysregulation of the gastric mucosal barrier

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### Abstract

Long-term chronic infection with *Helicobacter pylori* (*H. pylori*) is a risk factor for gastric cancer development. In the multi-step process that leads to gastric cancer, tight junction dysfunction is thought to occur and serve as a risk factor by permitting the permeation of luminal contents across an otherwise tight mucosa. Mechanisms that regulate tight junction function and structure in the normal stomach, or dysfunction in the infected stomach, however, are largely unknown. Although conventional tight junction components are expressed in gastric epithelial cells, claudins regulate paracellular permeability and are likely the target of inflammation or *H. pylori* itself. There are 27 different claudin molecules, each with unique properties that render the mucosa an intact barrier that is permselective in a way that is consistent with cell physiology. Understanding the architecture of tight junctions in the normal stomach and then changes that occur during infection is important but challenging, because most of the reports that catalog claudin expression in gastric cancer pathogenesis are contradictory. Furthermore, the role of *H. pylori* virulence factors, such as cytotoxin-associated gene A and vacuolating cytotoxin, in regulating tight junction dysfunction during infection is inconsistent in different gastric cell lines and *in vivo*, likely because non-gastric epithelial cell cultures were initially used to unravel the details of their effects on the stomach. Hampering further study, as well, is the relative lack of cultured cell models that have tight junction claudins that are consistent with native tissues. This summary will review the current state of knowledge about gastric tight junctions, normally and in *H. pylori* infection, and make predictions about the consequences of claudin reorganization during *H. pylori* infection.

**Key words:** *Helicobacter pylori*; Tight junction; Claudins; Paracellular permeability; Stomach; Cytotoxin-associated gene A; Vacuolating cytotoxin; Lipopolysaccharide; Urease; Ammonia

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**Core tip:** Tight junction dysfunction is a risk factor for cancer development during *Helicobacter pylori* (*H. pylori*) infection. The recent identification of numerous barrier-forming claudins has greatly improved our understanding of properties that regulate selective permeation across the tight junction in general, but little is known about the role of claudins in the stomach, or in *H. pylori* infection. In this article, we review the current state of knowledge on stomach tight junction composition and organization, discuss the details of claudin expression in various species and in cultured gastric cells, and discuss the implications of tight junction dysregulation in gastric cancer pathogenesis.

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## INTRODUCTION

Tight junctions have recently attracted a great deal of interest because of their regulated permeability to ions, solutes, and water conferred by a large and diverse group of transmembrane proteins consisting mainly of occludin, junctional adhesion molecules (JAM's), and members of the claudin family of proteins. Additionally, the discovery that increased intestinal permeability occurs during inflammation by tight junction dysfunction has driven new ways of thinking about the pathogenesis of inflammatory bowel diseases<sup>[1-3]</sup>. To date, the study of tight junction structure and function in the gastrointestinal tract has focused mainly on intestine and colon, which are considered "leaky" transporting epithelia that take advantage of tight junctions that are selectively permeable to ions and small molecules for passive paracellular absorption and secretion<sup>[4,5]</sup>. We propose that the stomach is a tighter epithelium that generates favorable ion gradients during active acid and pepsinogen secretion to drive passive, transcellular transport with little chance of cation (Na<sup>+</sup>, H<sup>+</sup>) movement across tight junctions. The stomach must act as a barrier to localize toxins, food substances, and the microbiota to the gastric lumen thus inhibiting access to the systemic circulation. The stomach has an additional challenge of limiting secretion-mediated hydrogen ion and pepsinogen back-diffusion across the epithelial barrier. *Helicobacter pylori* (*H. pylori*) infection and its resulting inflammation disrupt the mucosal barrier and thus pose a risk for gastric cancer development<sup>[6]</sup>. Despite the importance of an intact barrier in the stomach, little is known

about the physiology or function of tight junctions in gastric epithelial cells. Our focus in this article is to review current and past work on tight junctions in the stomach and to postulate on their role in disease pathogenesis and cancer development during *H. pylori* infection.

## ORGANIZATION OF HUMAN AND MOUSE STOMACH AND GASTRIC GLANDS

The human stomach is organized into four functional regions: (1) the cardia is localized as a ring of cells at the junction of the esophagus and stomach; (2) the fundus and (3) body (corpus) make-up the bulk of the stomach; and (4) the pylorus, consisting of the pyloric antrum and pyloric canal, is the most distal region located proximal to the duodenum (Figure 1). The mouse stomach has body and pylorus regions but additionally has an extensive forestomach consisting of squamous epithelial cells (Figure 1). Gastric glands in both the human and mouse stomach are present in all regions but differ in both cellular composition and in function; cardia and pylorus regions consist mostly of surface and gland mucous cells (not shown), whereas those in the fundus and body consist of surface epithelial cells facing the lumen, gastric pits, which contain mucous-secreting pit cells, and long glands that are further divided into the isthmus, neck, and base containing neck cells, parietal cells, and zymogenic (chief) cells respectively (Figure 2A). Numerous stem cells, committed progenitor cells, and endocrine cells also populate gastric glands in the fundus and body (Figure 2A). Although rarely denoted in schematic diagrams, the specialized epithelial cells in each region possess apical junctional complexes (Figure 2B-D) that consist of occluding and adherens junctions and desmosomes. While adherens junctions and desmosomes primarily function to regulate cell-to-cell adhesion and cell signaling, tight junctions regulate epithelial barrier function and paracellular permeability.

## TIGHT JUNCTIONS AND THE MUCOSAL BARRIER IN STOMACH

### *Tight junctions: General overview*

Tight junctions are multi-protein complexes composed of numerous transmembrane and cytoplasmic components that form a continuous structure around the lateral portion of epithelial cells near the luminal surface (Figure 3). By freeze-fracture microscopy analysis of the lateral cell membrane, the tight junction appears as linear rows of straight or anastomosing strands on the P-face (inner surface of the inner lipid monolayer), which likely represent integral membrane proteins of the tight junction, with corresponding grooves on the

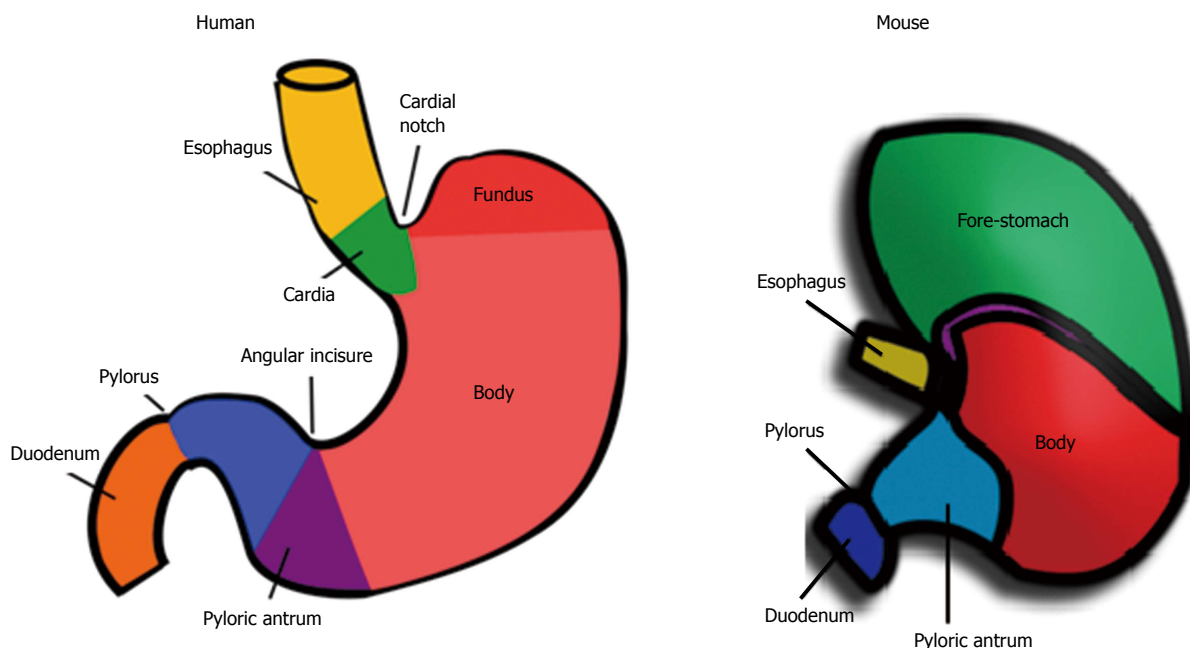


Figure 1 Gross anatomical characteristics of the human and mouse stomach.

E-face (inner surface of the outer lipid monolayer)<sup>[7]</sup>. The cell-specific strand number and network complexity may be important factors in regulating barrier properties<sup>[7]</sup>. The outer leaflet of the plasma membrane from adjacent cells additionally have kissing points, which are areas of the membrane that have virtually no intercellular space but rather contain regulated aqueous pores that are thought to function as passive ion channels<sup>[5,7,8]</sup>. Transmembrane proteins at the tight junction, including occludin and claudins, are associated with tight junction strands. Other transmembrane proteins found at tight junctions include tricellulin, marvelD3 and JAM proteins<sup>[9]</sup>. Transmembrane proteins are stabilized at tight junctions by peripheral scaffolding proteins such as zonula occludens (ZO)-1, -2, and -3, cingulin, afadin, membrane-associated guanylate kinase with inverted orientation-1 (MAG proteins) and multi-PDZ domain protein 1 (MUPP-1), which are linked to the actin cytoskeleton and to microtubules through numerous linker proteins like non-muscle myosins and cingulin; and a spectrum of associated signaling effectors are found in this macromolecular complex, like Rho, Rac, and cdc42<sup>[9]</sup>. For a comprehensive description of TJ components, see recent reviews by Van Itallie *et al.*<sup>[9]</sup> and Günzel *et al.*<sup>[10]</sup>.

#### **Tight junctions: Regulation of paracellular permeability**

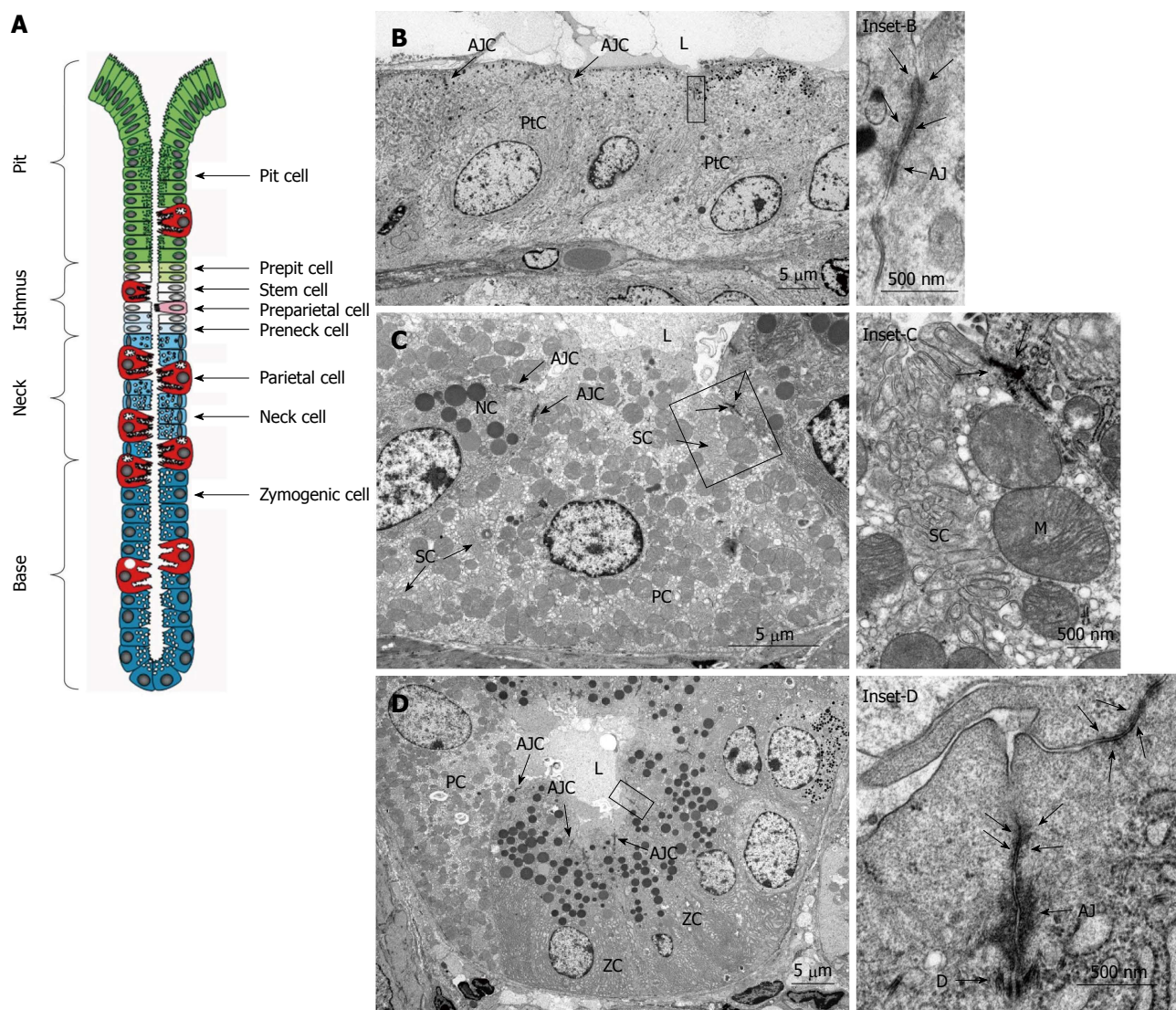
Two distinct pathways are involved in the regulation of paracellular permeability at tight junctions. The first pathway is the “pore” pathway, which allows the movement of small molecules, ions, and nutrients through the tight junction along with water. The pore pathway (1) allows charged or uncharged molecules less than approximately 4 angstroms (Å) to cross the tight junction with charge discrimination that is

regulated by the expression of claudin molecules; (2) carries most of the electrical current for a given epithelium (reflected in the measurement of transepithelial (electrical) resistance, TER); and (3) regulates the magnitude of permeability and charge selectivity as determinants of tissue-specific physiological transport properties<sup>[11]</sup>. The second pathway, or “leak” pathway, allows the flux of molecules larger than 4 Å across the tight junction with no charge selectivity that may be due to small temporary breaks in otherwise continuous tight junction strands<sup>[11]</sup>. This pathway is controlled by cytoskeletal dynamics or factors that affect cell homeostasis<sup>[11]</sup>. Reports that describe barrier dysfunction in *H. pylori* infection suggest that both pathways are affected; *H. pylori* infection (1) decreases TER and increases permeability, thus affecting the pore pathway including the expression of claudin molecules, claudin composition at tight junctions, and the magnitude of paracellular flux; (2) causes small breaks in tight junction strands and thus increases the flux of sucrose (5.2 Å) and other molecules during infection; and (3) injures gastric epithelial cells, which not only disrupts tight junctions but lateral membrane adherence, in general. The details of each will be discussed below.

#### **Tight junctions: Molecular architecture in gastric epithelial cells**

Tight junction structure specific to various parts of the stomach or specific to individual epithelial cell types in the stomach have not been well-characterized. Claude and Goodenough<sup>[12]</sup> originally classified the mouse stomach as “very tight” because tight junctions had the same number of strands (range, 5-14 strands) described in the urinary bladder, which had a high

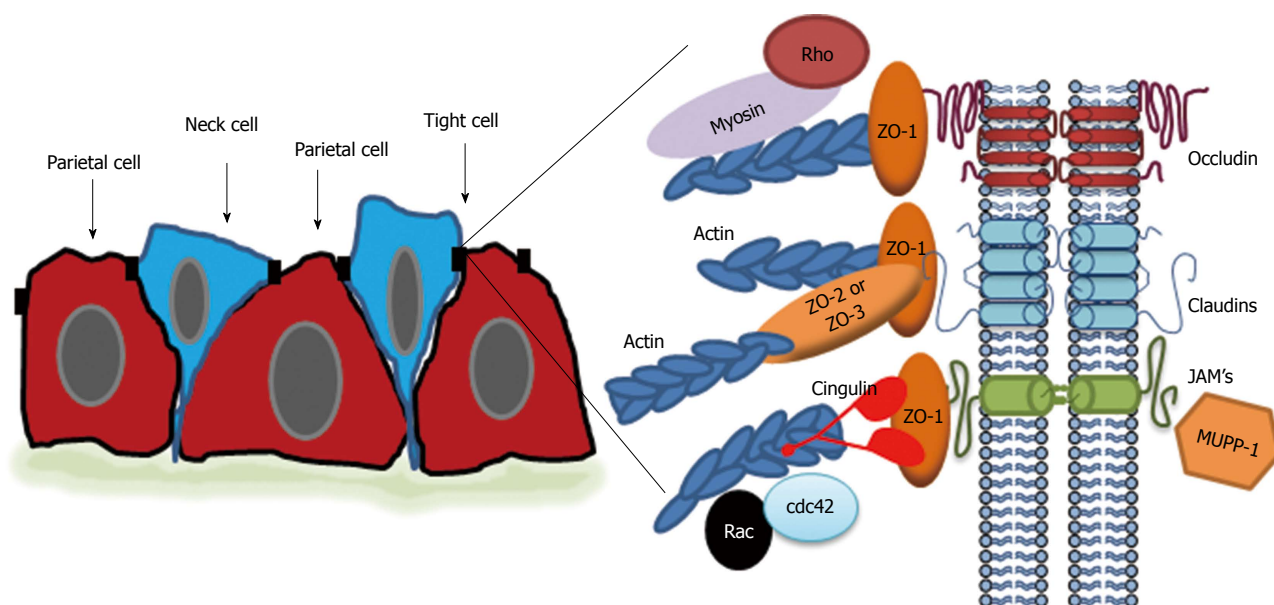




**Figure 2** Histological structure of a gastric unit. A: Diagrammatic representation of the organization of a gastric unit (also called a gastric gland), which contains a pit, isthmus, neck, and base. The location-specific cell types are identified in each region. Reproduced with permission from Karam SM<sup>[109]</sup>; B: Pit region cells (PtC) have apical junctional complexes (AJC) that are near the gastric lumen (L). Inset from the box in B: contains a tight junction (arrows) and an adherens junction (AJ). The desmosome is out of plane in this image. Bar in B is 5 μm and in the inset is 500 nm; C: Neck region cells, consisting mainly of parietal cells (PC) and neck cells (NC) also have AJC near the lumen of the gastric gland (L). Secretory canaliculi (SC) and mitochondria (M) are prominent in parietal cells. Inset from the box in C: contains a tight junction (arrows) and other parts of the apical junctional complex that are out of plane. Bar in C is 5 μm and in the inset is 500 nm; D: Base region cells consist mostly of zymogenic cells (ZCs) and a few PCs that also have AJC. Note that similar to the diagram in A, the apical cell cytoplasm of the zymogenic cells extends as a triangular wedge into the gland lumen (L) and apical junctional complexes are found at the lateral membranes where cells meet. Inset from the box in D: contains an apical junctional complex consisting of the tight junction (arrows), AJ, and desmosome (D). Bar in D is 5 μm and in the inset is 500 nm.

TER of 1000-2000 Ohm·cm<sup>2</sup>. Structural differences were then described in tight junction strands in various parts of the gastric unit<sup>[13]</sup>, suggesting that the tightness and transport properties of tight junctions are different at the surface and in gastric glands. As an example, tight junctions in surface epithelial cells are composed of 5 to 6 strands that are woven together into a deep, honeycomb-type structure whereas the same number of strands in cells from gastric glands (both parietal and chief cells) are organized in a shallow, regular, linear configuration<sup>[13]</sup>. Of particular note, however, was the difference in permeability in the two regions. When lanthanum (La<sup>3+</sup>) was instilled into the gastric lumen during fixation, this small (4.2

Å) electron-dense molecule was unable to cross tight junctions and was thus excluded from the basolateral intercellular space between adjoining surface epithelial cells<sup>[13]</sup>. In contrast, La<sup>3+</sup> was frequently found within the basolateral membrane space in gastric glands, particularly surrounding parietal cells<sup>[13]</sup>. Another novel finding specific to stomach were structures resembling tight junctions along the basolateral membrane of epithelial cells by freeze fracture microscopy<sup>[12]</sup>; the structures described were discontinuous and were proposed to be unrelated to the regulation of epithelial permeability<sup>[12]</sup>. Overall, these interesting findings suggests that epithelial cells in gastric glands, compared to surface epithelial cells, are particularly permeable to



**Figure 3** Schematic representation of the tight junction in gastric epithelial cells. In the neck region of gastric glands, neck and parietal cells are oddly shaped but make tight junctions at the apical border between cells. Expanded diagram: Tight junctions in the stomach have classical components consisting of transmembrane proteins including occludin, claudins, and JAM proteins; peripheral scaffolding proteins like zonula occludens (ZO)-1, 2, and 3; linker proteins to the actin cytoskeleton like non-muscle myosin and cingulin; and signaling molecules like Rho, Rac, and cdc42. Actin filaments are also prominent.

small ions due to the composition and organization of tight junction strands, and that novel, tight junction-like structures may also be a feature of the basolateral membrane of gastric epithelial cells.

Gastric epithelial cells contain transmembrane proteins, like occludin, JAM-A, and claudins<sup>[14-19]</sup>, and peripheral scaffolding proteins, like ZO-1<sup>[17]</sup> (Figure 3). In the human stomach, immunostaining for occludin appeared to localize at the tight junction and along the basolateral membrane<sup>[16]</sup>, but because there was no accompanying control to evaluate non-specific staining it is not clear if this result truly reflects the localization of occludin *in vivo*. Tricellulin has been localized to tricellular contacts within tight junctions in epithelial cells of human stomach<sup>[16,20]</sup>. There was also considerable lateral membrane staining for tricellulin; however, without controls for non-specific background it is not clear whether the lateral membrane staining is specific.

Little is known about the differential expression of claudin proteins in tight junctions of normal gastric epithelial cells. Because claudins localize to tight junction strands and because the strand configuration is different in surface cells compared to the cells in gastric glands, it may be important to determine the claudin footprint of each cell-type or area to better understand the details of permselectivity in the stomach.

**Claudins and selective permeability or “permselectivity”**

Although the “tightness” or “leakiness” of tight junctions was originally proposed to be determined by the number and depth of tight junction strands<sup>[7,12]</sup>, knowledge about the presence of tight junction

claudins currently dominates our thinking about the regulation of tight junction permeability and permselectivity. Although occludin is expressed in stomach epithelial cells and is membrane-spanning at tight junctions, this protein is not involved in the regulation of paracellular permeability in the stomach<sup>[15]</sup>. In contrast, claudin proteins are expressed in the stomach and are likely to determine epithelial permeability and permselectivity.

In mammals, the claudin family of proteins currently consists of 27 different tetraspanning proteins that are normally expressed in a tissue dependent fashion<sup>[10,21]</sup>. Claudins associate with a host of other cytoplasmic and extracellular proteins, and play roles in the regulation of tight junction permeability, cell signaling, cell cycle regulation, the maintenance of cell polarity, and vesicle trafficking<sup>[10]</sup>. The crystal structure of mammalian claudin 15 was recently determined, revealing four underlying transmembrane helices which anchor a unique extracellular beta-sheet fold made from the first and second extracellular loops<sup>[8,22]</sup>. These extracellular loops contain 5 beta strands which, when aligned properly into 2 continuous antiparallel rows, have been proposed to form “half-pore” structures, each containing two variable regions which, when aligned with adjacent cells, form complete TJ pores<sup>[8]</sup>. The model, which was described by Suzuki *et al.*<sup>[8]</sup>, suggests that the charges possessed by two intrapore variable regions in each claudin dimer determine the selective permeability characteristics. Just as the specific claudin populations expressed in different epithelial cell types are thought to determine tissue specific solute permeability, altered claudin expression



has been linked to a number of pathologic conditions including gastric cancer (discussed below).

### **Claudin expression in the stomach-an overview**

**Human stomach:** By genetic analysis using the serial analysis of gene expression (SAGE) database followed by RT-PCR techniques, the expression of claudins 1-5, 7-12, 16 and 18 have been demonstrated in normal human stomach (Table 1). Bioinformatics approaches combined with genome-wide analyses also identified claudins 21-23 in the human stomach (Table 1).

Immunostaining analysis in human stomach demonstrated the expression of claudin 1, 3-5, 7, 10, 14, and 18 (Table 1). Claudin 1 expression was high in epithelial cells from both corpus and antrum, whereas the expression of claudins 3, 4, and 5 was stronger in corpus compared to antrum (Table 1). In other work, immunostaining analysis of human tissues concluded that weak to no claudin 3 or 4 expression was present in the normal gastric mucosa (Table 1). Normal stomach tissues adjacent to gastric tumors demonstrated claudin 1 expression in about 50% of tissues, claudin 3 expression in about 24% of tissues, and claudin 4 expression in about 15%<sup>[23,24]</sup> to as high as 40%-50% of tissues<sup>[17,25,26]</sup>. Similarly, claudins 2, 6, and 11 were expressed in 68%, 79% and 46% of tissues, respectively<sup>[27]</sup>. These results suggest that the differing results in studies from human patient gastric samples might be explained, at least in part, by the source and/or location of "normal" tissues used in for immunostaining. As for location and cell specificity in human studies, claudin 1 was found to be strongly expressed in gastric surface epithelial cells and chief cells whereas it was weakly expressed in parietal cells (Table 1). Other than the localization of claudin 18 (below), the localization of other claudins to specific epithelial cell types in the corpus or antrum is unknown (Table 1).

**Canine stomach:** A recent immunohistochemical analysis of claudin expression in the normal canine stomach revealed a robust basolateral membrane localization of claudin 18 in all fundic epithelial cells<sup>[28]</sup>. In the pylorus, all glandular cells expressed claudin 18, while only basally located glandular cells expressed claudin 2<sup>[28]</sup>. Surface, mucous neck, parietal, chief, and endocrine cells of the fundus, as well as surface and glandular cells in the pylorus were negative for claudins 1, 3-8 and 10<sup>[28]</sup>.

**Rat stomach:** Immunohistochemical analysis of claudins 2, 3, 4, and 5 expression in Sprague Dawley rats showed that there was no difference in the expression level or cellular localization of these specific claudin in any region of the stomach; claudin 3 was most highly expressed at the basolateral membrane of surface epithelial cells, without enrichment at the tight junction<sup>[29]</sup>. Similarly, claudin 5 was localized to the basolateral membrane of all cells comprising

the gastric glands<sup>[29]</sup>. The only tight junction protein identified to localize at the tight junction *per se*, with no basolateral expression, was claudin 4, which also showed higher expression in proximal gastric glands<sup>[29]</sup>. Claudin 2 expression was not detected in any part of the stomach<sup>[29]</sup>.

**Mouse stomach:** By quantitative RT-PCR, claudins 1, 3, 4, 6, 7, 10, 12, 15, 17, 18, 23, and 25 are expressed in the stomach of neonatal C57BL/6 mice<sup>[14]</sup>. By immunostaining, claudins 1, 3, 5, and 18 are expressed in the glandular stomach while claudins 6 and 11 are confined to the squamous fore-stomach<sup>[30]</sup>. Claudins 1 and 3 are localized to the basolateral membrane of epithelial cells in the glandular stomach whereas claudins 5 and 18 are basolateral but appear to be enriched at tight junctions<sup>[30]</sup>. In the stomach from adult C57BL/6 mice, low levels of all claudin-family members were present, as determined by quantitative RT-PCR analysis<sup>[14,31]</sup>, but claudin 18 was expressed at a level considerably higher than all other claudin-family members<sup>[14]</sup>. Immunostaining studies in adult mouse stomach consistently showed that claudin 2 expression was negative in corpus epithelial cells but present at the base of antral glands<sup>[32]</sup>, similar to the canine stomach<sup>[28]</sup>.

### **Claudin 18 expression in gastric epithelial cells**

Claudin 18 is likely to be the most important barrier-forming claudin family member in the stomach because its expression is 30-fold or more greater than all other claudins, at least in mouse stomach<sup>[14]</sup>. In general, claudin 18 has four differentially expressed isoforms; claudin 18A1.1 and A1.2 are expressed almost exclusively in lung and claudin 18A2.1 and 18A2.2 are expressed almost exclusively in the stomach, with claudin 18A1.1 highly expressed and claudin 18A.2 barely present<sup>[33]</sup>. Additionally, claudin 18A2.1 localizes to the basolateral membrane of gastric epithelial cells rather than being concentrated solely at tight junctions<sup>[14,33,34]</sup>. When transfected into Madin-Darby canine kidney epithelial (MDCK) cells, claudin-18 raised electrical resistance and significantly reduced the paracellular permeability to cations, specifically Na<sup>+</sup> and H<sup>+</sup><sup>[35]</sup>, suggesting that claudin-18 functions as a strong cation exclusion pore at tight junctions. Knockout mice (C57BL/6) deficient in *C18A2.1* confirmed the importance of this claudin in stomach, given the knockout mice displayed an increase in paracellular H<sup>+</sup> leakage as well as transepithelial conductance. Additionally, inflammation was present and the mice rapidly developed atrophic gastritis due, in part, to H<sup>+</sup> back-diffusion and mucosal injury<sup>[14]</sup>. In human studies (Table 1), the attenuation of claudin 18 expression in the gastric mucosa was prominent in early GC development, and predicted an unfavorable outcome after cancer diagnosis<sup>[34,36-38]</sup>. Although these studies suggest that a loss of gastric epithelial claudin 18 leads to the stepwise development

**Table 1 Claudin expression in the normal human stomach and changes that occur in gastric cancer**

	Location	Detection method	Expression (normal stomach)	Patient outcome	Changes in GC
Cldn 1	Unspecified region	SAGE database and RT-PCR <sup>[110]</sup>	Present	Not evaluated	Present, no change in GC
	Unspecified region	cDNA oligonucleotide microarray analysis <sup>[67]</sup>	Present	Up-regulation results in extremely poor outcome	One of the most highly up-regulated genes
	Corpus, antrum	Immunostaining <sup>[69]</sup>	Strong expression in epithelial cells	No association with patient outcome	Some GC with strong expression and some with no expression
	Antrum	Immunostaining <sup>[74]</sup>	Strong expression in epithelial cells	No association with patient outcome	No change in expression in GC
	Unspecified region	Immunostaining <sup>[68,70]</sup>	Not evaluated	Not evaluated	Highly expressed in GC; most highly expressed at invasive front
	Unspecified region	Immunostaining <sup>[25]</sup>	Tumor margin	Not evaluated	55.4% of cells are positive at the tumor margin Reduced expression in GC
	Corpus	Immunostaining <sup>[73]</sup>	Surface and chief cells +++; parietal cells +	Not evaluated	Basolateral localization Expression in GC is dependent on the expression of RUNX3 No change in GC
Cldn 2	Unspecified region	qRT-PCR <sup>[111]</sup>	Weak expression	Not evaluated	Highly up-regulated in GC
	Unspecified region	cDNA oligonucleotide microarray analysis <sup>[67]</sup>	Present	Not evaluated	Highly expressed in GC
	Unspecified region	Immunostaining <sup>[68,83]</sup>	Not evaluated	Not evaluated	Highly expressed in GC
Cldn 3	Unspecified region	SAGE database and RT-PCR <sup>[110]</sup>	Present	Not evaluated	Up-regulated in GC
	Unspecified region	Immunostaining <sup>[112]</sup>	Not evaluated	Not evaluated	Higher expression in low grade compared to high-grade malignancy
	Unspecified region	Immunostaining <sup>[25,36,68,113-116]</sup>	Low to no expression in stomach	Up-regulation has no effect on survival	Highly expressed in the majority of GC's
	Corpus, antrum	Immunostaining <sup>[69]</sup>	Corpus, strong expression; Antrum, weaker expression	Up-regulation associated with a significantly higher incidence of synchronous and metachronous multiple GC and gastric adenomas <sup>[114]</sup> Strong expression results in better outcome.	Increase in expression occurs in metaplasia Some GC with strong expression and some with no expression
Cldn 4	Antrum	Immunostaining <sup>[74]</sup>	No expression	No association with patient outcome	Most GC weak to moderate expression
	Unspecified region	SAGE database and RT-PCR <sup>[17,110]</sup>	Present	Not evaluated	Highly up-regulated in GC
	Unspecified region	Immunostaining <sup>[112]</sup>	Not evaluated	Not evaluated	Higher expression in low grade compared to high-grade malignancy
	Unspecified region	Immunostaining <sup>[17,23-26,68,113-117]</sup>	Low to no expression in stomach	No association with patient outcome.	Highly expressed in GC Localized to the basolateral membrane Prominent in intestinal-type GC
	Unspecified region	Immunostaining <sup>[126]</sup>	Low to no expression in stomach	High expression is associated with favorable prognosis and longer survival; low expression is associated with poor survival	Highly expressed from stages intestinal metaplasia to GC Localized to the basolateral membrane
	Corpus, antrum	Immunostaining <sup>[69]</sup>	Corpus, strong expression; Antrum, weak expression	No association with patient outcome	Strong expression associated with metaplasia Some GC with strong expression and some with no expression
	Antrum	Immunostaining <sup>[74]</sup>	No expression	High expression associated with poor outcome	Intestinal metaplasia highly expressed 90% of GC have weak to strong expression



Cldn 5	Unspecified region Corpus, antrum	SAGE database and RT-PCR <sup>[110]</sup> Immunostaining <sup>[69]</sup>	Present Corpus, strong expression; Antrum weak expression.	Not evaluated No association with patient outcome.	Present, no change in GC Some GC with strong expression and some with weak expression.
Cldn 7	Unspecified region	SAGE database and RT-PCR <sup>[110]</sup>	Present	Not evaluated	Highly up-regulated in GC
	Unspecified region	cDNA oligonucleotide microarray analysis <sup>[67]</sup>	Present	Not evaluated	Highly up-regulated in GC
	Unspecified region	Immunostaining <sup>[36]</sup>	Not present in stomach	Up-regulation correlated with poor survival	Highly up-regulated in GC
Cldns 8-12	Unspecified region	SAGE database and RT-PCR <sup>[110]</sup>	Present	Not evaluated	Present, no change in GC
Cldn 10	Unspecified region	Immunostaining <sup>[118]</sup>	Highly expressed	Not evaluated	Significantly reduced in GC
Cldn 11	Unspecified region	RT-PCR methylation analysis <sup>[119]</sup>	Not evaluated	Not evaluated	Highly methylated in gastric cancer, which is correlated to attenuated expression.
Cldn 14	Unspecified region	Immunostaining <sup>[118]</sup>	Little to no expression	Not evaluated	Highly expressed in GC Localization to the basolateral membrane
Cldn 16	Unspecified region	SAGE database and RT-PCR	No expression	Not evaluated	No expression in GC
Cldn 17	Unspecified region	Immunostaining <sup>[118]</sup>	Highly expressed	Not evaluated	Significantly reduced in GC
Cldn 18	Unspecified region	SAGE database and/or RT-PCR <sup>[110,34,36]</sup>	Not evaluated	Down-regulation correlated with poor survival	Identified as a highly expressed gene that is significantly down-regulated in GC
	Unspecified region	RT-PCR <sup>[34,120]</sup>	Not evaluated	Not evaluated	Cldn18A1 is not expressed in stomach or in GC whereas Cldn18A2 is expressed in stomach and in some GC's Basolateral localization.
	Corpus or antrum	Immunostaining <sup>[34,37,113]</sup>	Surface, ++++ Pit, + Parietal/Neck, +++ Zymogenic, +++	Down-regulation correlated with poor survival	Attenuation is an early event, which occurs in the metaplastic mucosa
Cldns 21, 22, 23, 24	Database search	Bioinformatics <sup>[121]</sup>	Not evaluated	Not evaluated	Identified genes for Cldns 21-24
Cldn 23	Unspecified region	Genome-wide analysis <sup>[122]</sup>	Not evaluated	Not evaluated	Cldn-23 down-regulated in 78.9% of GC with an intestinal phenotype

++++, very highly expressed; +, weak expression. Cldn: Claudin; GC: Gastric cancer.

of chronic inflammation and gastric cancer, it has not been shown that the expression of claudin 18 is affected by *H. pylori* infection nor has it been shown that the attenuation of claudin 18 results in cancer development. Thus, direct cause-and-effect evidence is lacking. Additionally, it is possible that other claudins with similar cation-exclusion function might be upregulated to compensate for the lack of claudin 18 expression in GC development.

## HELICOBACTER PYLORI: DISEASE PARAMETERS THAT AFFECT BARRIER DYSFUNCTION - OVERVIEW

*H. pylori* is a highly adapted, spiral shaped, gram negative bacteria that colonizes the human stomach, with animal-adapted cultivars that infect the non-human primate, cat, mouse, guinea pig, gerbil, and rat stomach<sup>[39]</sup>. The bacterium is catalase and oxidase positive, microaerophilic, and possesses 3 to 5 polar sheathed flagella that are used for motility<sup>[40]</sup>. *H.*

*pylori* also possess urea transporters that are utilized during acid exposure, in conjunction with urease, to neutralize pH, and support colonization<sup>[41,42]</sup>. Both *H. pylori* and ammonia have been identified as important factors that regulate barrier dysfunction in *H. pylori* infection, the details of which can be found in the section on cultured cells, below. *H. pylori* are also associated with a specific set of virulence factors, including VacA and the cytotoxin-associated gene pathogenicity island (Cag PAI), which codes for a type 4 secretion system that delivers CagA into the cytoplasm of epithelial cells. Both VacA and CagA are considered important *H. pylori* virulence factors that regulate disease pathogenesis and barrier dysfunction. The role of VacA and CagA on *H. pylori*-induced barrier dysfunction is also discussed in detail below in the section on cultured cells. Lipopolysaccharide (LPS) from *H. pylori* is also considered toxic to gastric epithelial cells<sup>[43]</sup>. While *H. pylori* LPS is considered less potent than the LPS from other bacteria, like *E. coli*<sup>[43]</sup>, it decreases the TER and increases the permeability of cultured primary gastric

epithelial cells<sup>[44]</sup>. Interestingly, this effect is greater from the basolateral compared to apical surface<sup>[44]</sup> suggesting that LPS may be most effective at damaging the mucosal barrier if it is able to permeate the mucosa and gain access to the basolateral surface of gastric cells.

*H. pylori* is categorized as a type 1 carcinogen by the World Health Organization and International Agency for Research on Cancer<sup>[45]</sup>, and is responsible for a large percentage of gastric cancer, which is the fifth most common cancer and the third most common cause of cancer-related deaths worldwide<sup>[46,47]</sup>. Without therapeutic intervention, *H. pylori* infection leads to a persistent, life-long infection. *H. pylori* infection of the gastric corpus is associated with intestinal-type gastric adenocarcinoma and initiates a well-defined pathological process, referred to as the "Correa Cascade", which is characterized by chronic superficial gastritis followed by atrophic gastritis and intestinal metaplasia, which progresses to dysplasia and adenocarcinoma<sup>[48,49]</sup>. Although eradication of *H. pylori* appears to be the most feasible approach to reducing GC rates, previous studies were recently reviewed by Lu *et al.*<sup>[50]</sup> and suggest otherwise. Furthermore, eradication of *H. pylori* after endoscopic resection of tumors does not reduce the development of metachronous gastric carcinoma<sup>[51]</sup>, suggesting that either *H. pylori*- or inflammation-induced genetic and epigenetic changes in gastric epithelial cells, microsatellite instability, or other permanent changes occur in the stomach that cannot be reversed by bacterial eradication. Changes in the expression of tight junction components may be part of this global pattern of gene expression changes that impact cancer pathogenesis.

## DISRUPTION OF TIGHT JUNCTIONS IN *H. PYLORI* INFECTION

Early studies using electron microscopy and human biopsy samples from patients infected with *Campylobacter pylori*, the organism later renamed *H. pylori*, demonstrated that *C. pylori* colonize the gastric mucosa and are highly concentrated along the luminal surface of surface mucous cells proximal to tight junctions<sup>[52]</sup>. They also migrate between epithelial cells<sup>[53]</sup>. The bacteria target surface epithelial cells that express *Limax flavus* agglutinin, which is a lectin that is specific for sialic acid-rich glycoproteins, and adhere to the surface by making intimate contacts that result in the depletion of microvilli and alterations in the cell including dissolution of apical secretory granules and rounding of the apical cell surface<sup>[52]</sup>. *C. pylori* were also found to penetrate the apical junctional complex<sup>[53]</sup>. Later Necchi *et al.*<sup>[54]</sup>, using tissues from *H. pylori* infected patients, confirmed these initial findings and extended them to show that junctional penetration occurs in both antrum and corpus, and

demonstrated that although most of the tight junctions were intact, there were some cells with detached junctional complexes that contained CagA<sup>+</sup>VacA<sup>+</sup> *H. pylori* traversing the intercellular space and residing near the base of cells. Noach *et al.*<sup>[55]</sup> described that the majority of *H. pylori* were scattered in the mucous layer or positioned at the aforementioned tight junction location without cell contact but some bacteria formed adhesion pedestals at the bacterial and cell interface and were seen entering cells, which occurred next to tight junctions. Further work, reviewed by Fox *et al.*<sup>[56]</sup>, described that most of the bacteria in the infected stomach exist in a non-adherent configuration in the extracellular mucous environment. By freeze fracture microscopy, uninfected antral epithelial cells had a thin tight junction area consisting of about four strands that formed interconnecting ridges whereas the tight junction region from *H. pylori*-infected patients was significantly deeper with irregular, knobby, and locally fragmented strands<sup>[55]</sup>. Tannic acid, which was used to evaluate live and dead cells in tissues from *H. pylori*-infected patients, demonstrated that many of the cells from infected vs control patients were tannic acid-positive, but additionally, the number of tannic acid-containing cells was associated with the inflammatory score of the specimen<sup>[57]</sup>. Sucrose permeability and the permeability of food antigens increased in patients with *H. pylori* infection<sup>[58-60]</sup>, supporting the notion that barrier dysfunction accompanies defects in tight junction structure during infection. Animal models also support this idea; C57BL/6 mice infected with *H. pylori* Sidney strain 1 (SS1) showed a 30-fold increase in lanthanum (4.2 Å) permeability across tight junctions into the intercellular space *in vivo*<sup>[61]</sup>, and an increase in sucrose (4.6 Å) excretion, *in vivo*, was also found in infected mice<sup>[62]</sup>. In the later study, permeability defects occurred only transiently, at about 12 wk post-infection, whereas permeability before 12 wk and up to 100 d post-infection was not significantly different from control mice<sup>[62]</sup>. The reason for this result is unknown. In *H. felis*-infected C57BL/6 mice, Ussing chamber experiments were used to demonstrate that infected mice also increased antral HRP flux (30 Å)<sup>[63]</sup>. Overall, these results suggest that both tight junction pore and leak pathways are affected during *H. pylori* infection, resulting in an increase in small molecule permeation across tight junctions and an increase in the permeation of larger molecules that may be due to small temporary breaks in tight junction strands. Alternatively the leak pathway alone may be activated by defects in cytoskeletal dynamics, particularly MLCK activity, consistent with the results obtained in cultured cells by Wroblewski *et al.*<sup>[64]</sup>. Interestingly, HRP flux across tight junctions by electron microscopy correlated with transitional zone neutrophilic gastritis<sup>[61]</sup> and eradication of *H. felis* infection in C57BL/6 mice returned the HRP permeability defect to normal if inflammation concomitantly decreased<sup>[63]</sup>. These results suggest that inflammation may be the most important

component of tight junction and permeability defects in *H. pylori* infection.

### **Inflammation and gastric tight junction dysfunction**

Few studies have been done to address the role of inflammatory cytokines on tight junction dysfunction in the stomach or in model gastric epithelial cells. Gastric HGE-20 cells were initially used to show that IL-1 receptor phosphorylation by interleukin (IL)-1 $\beta$  occurs after exposure to *H. pylori*, resulting in the reduction of claudin 4 expression that seems to be internalized into the cell cytoplasm<sup>[65]</sup>. This work was not accompanied, however, by TER or permeability studies, so it is unclear whether or not exposure to IL-1 $\beta$  results in barrier dysfunction or just targets specific claudins for degradation. It is also unclear how IL-1 receptor phosphorylation occurred after exposure to *H. pylori* in cultured cells because immune cells were not present in the assay; whether some aspect of infection-mediated signaling activated the receptor pathway or if the cells secreted IL-1 $\beta$ , which self-activated the receptor. To address this issue using NCI-N87 cells, Fiorentino *et al*<sup>[66]</sup> showed that exposure to *H. pylori* reduced TER and increased paracellular permeability over time without a reduction in cell viability but with a concomitant increase in cytokine production by epithelial cells, including IL-8, IL-6, interferon (IFN)- $\gamma$ , IL-1 $\beta$ , tumor necrosis factor (TNF)- $\alpha$ , and IL-10, which increased with either live or heat-killed bacteria. On the other hand, barrier dysfunction was accompanied by the reorganization of ZO-1 and claudin 1 proteins, but required live bacteria<sup>[66]</sup>. Thus, a cause and effect relationship between cytokine production and barrier defects in NCI-N87 cells was not fully established nor was the role of any particular cytokine further investigated to determine which would be involved in barrier dysfunction. These preliminary studies, in addition to the seminal studies on permeability in human and animal models, provide justification for further studies examining the role of pro-inflammatory cytokines in modulating tight junction dysfunction during infection. It would be very interesting to determine, in particular, if inflammation modulates the expression of claudin molecules that are normally responsible for maintaining the gastric mucosal barrier.

### **Claudin protein expression in *H. pylori* infection-mediated GC**

There has been considerable work done to catalog the changes in claudin expression in human gastric cancer caused by *H. pylori* infection (Table 1). These results, however, are difficult to interpret because the results from different groups, who are evaluating the expression of the same claudin, may be completely opposite, making it difficult to elucidate the role of claudins in cancer pathogenesis. As an example, Claudin 1 expression (Table 1) was found to be significantly lower in GC tissue vs adjacent tissues with no correlation to histological grade in one study<sup>[25]</sup>, but in another study it was highly expressed in GC,

as one of the most differentially upregulated genes in gastric tumors vs control tissues<sup>[67]</sup>. Similarly, in one study (Table 1) claudin 1 expression was more prominent overall in diffuse compared to intestinal GC<sup>[68]</sup> but in another study showed less expression in diffuse vs intestinal-type GC<sup>[69]</sup>. Claudin 1 expression was reported to be highest at the invasive front of GC, being highest in well-to-moderately differentiated carcinomas and lowest in poorly-differentiated carcinomas<sup>[70]</sup>. These studies were consistent with some cell culture experiments (Table 1), which demonstrated that over-expression of claudin 1 increased the migration and invasion of cultured gastric cells<sup>[71,72]</sup>, but in contrast to others (Table 1), which demonstrated that the attenuation of claudin 1 increased migration and invasion<sup>[73]</sup>. The later study went-on to conclude that normally high expression levels of claudin 1 function as a tumor suppressor<sup>[73]</sup>. In some studies (Table 1), the expression of claudin 1 was not associated with patient outcome<sup>[69,74]</sup>, whereas in another report it was highly correlated to patient outcome, with cumulative survival rates of 0% at about 12 postoperative months for patients with high claudin 1 expression vs about 50% survival at 50 mo for patients with low claudin 1 expression<sup>[67]</sup>. Each study included a significant number of patients, had control staining to verify the antibody, but found significantly different results. For patient studies to be meaningful, it appears that there must be guidelines adopted for study design to avoid conflicting results. What seems to be consistent between studies is that claudins 2, 3, 4, and 7 are highly upregulated, whereas claudin 18 is down-regulated in gastric cancer (Table 1). Recent studies have also evaluated less well-known claudins, such as claudins 10, 11, 14, 17, and 23 and found that claudins 10, 11, 17, and 23 were down-regulated and claudin 14 was highly up-regulated in patients with gastric cancer (Table 1). For a comprehensive review of claudin expression in gastric carcinogenesis, see Irvani *et al*<sup>[75]</sup>.

Claudin expression changes in GC, including the attenuation of claudins 11 and 18, which normally produce a tight barrier<sup>[11]</sup>, and an increase in claudin 2, which would significantly increase the paracellular cation leak<sup>[11]</sup>, suggest that the paracellular barrier would be leaky in GC tissues, particularly to cations like Na<sup>+</sup> and H<sup>+</sup>. This may be particularly detrimental in the stomach, which normally functions to limit H<sup>+</sup> back-diffusion from the lumen but furthermore, must maintain an effective luminal to basolateral Na<sup>+</sup> gradient so that ion transport functions can occur for pH regulation and for H<sup>+</sup> and bicarbonate secretion. It is possible that the upregulation of claudins 3, 4, 7, and 14, which function to tighten the barrier<sup>[11]</sup>, and in particular claudin 14, which forms a strong cation exclusion channel<sup>[5]</sup>, may increase as a means to compensate for the attenuation of claudin 18; in an attempt to regain barrier function and permselectivity in the absence of claudin 18. Claudins are also

known to have other functions in addition to acting at tight junctions to regulate permeability. Claudin 7, which is highly upregulated in GC progression in both humans (Table 1) and in *Helicobacter felis*-infected insulin-gastrin (INS-GAS) transgenic mice (FVB/N background)<sup>[76]</sup>, may function in a protective manner to regulate ion transport and regain NaCl homeostasis as described for intestine<sup>[77]</sup>, or it may drive tumorigenesis by binding to epithelial cell adhesion molecule (EpCAM) and regulating cancer pathogenesis<sup>[78,79]</sup>. Further studies would be required to resolve these two interesting possibilities.

## STUDYING *H. PYLORI*-INDUCED TIGHT JUNCTION DYSFUNCTION IN CULTURED GASTRIC AND OTHER CELLS

One challenge in studying gastric barrier function in reductionist models is the lack of gastric-specific cell lines that form a confluent monolayer with a robust luminal to serosal permeability barrier. Most of the cell lines available for studying the gastric mucosal barrier lack or do not have a completely profiled inventory of TJ components, do not grow in monolayers, and do not express claudin 18 (Table 2). The human NCI-N87 gastric cancer cell line is one exception, in that it forms a confluent cell monolayer, expresses claudin 18 (Table 2), and has a transepithelial resistance (TER) of about 1000 Ohm cm<sup>2</sup><sup>[66]</sup> compared to MKN28 cells, which can be induced to form a confluent monolayer but without a significant TER<sup>[64]</sup>. Clones isolated from NCI-N87 cells, in particular the HGE-20 clone (Table 2), grow in a confluent monolayer that is polarized, have apical junctional complexes that express ZO-1, express some markers of prezygogenic cells, and have a TER of about 200 Ohm cm<sup>2</sup><sup>[80,81]</sup>. Gastric adenocarcinoma AGS cells form a confluent monolayer that express ZO-1 and numerous claudins (Table 2), but lack the ability to form functional TJs when grown in monolayers. Because of these important challenges, AGS cells are often used in conjunction with other cells lines for studies concerning gastric barrier function<sup>[62,66,82-84]</sup>. Limited by the lack of appropriate gastric cell lines, most studies have used intestinal, colonic, or kidney cell lines, including SCBN cells<sup>[66]</sup>, MDCK cells<sup>[84,85]</sup>, T-84<sup>[86]</sup>, or Caco-2<sup>[58,87]</sup> cells to unravel mechanisms related to the role of *H. pylori* in barrier dysfunction. Gastric organoids from human biopsy samples<sup>[88]</sup> and primary human cultured cells from biopsy samples<sup>[89,90]</sup> are also viable options; they each form a monolayer of native gastric epithelial cells and have been shown to express occludin or ZO-1 at cell junctions. However, the compliment of other tight junction components including claudins has not been determined.

**CagA:** Disruption of the tight junction complex with CagA was first studied in MDCK cells, which clearly demonstrated TJ disruption including relocation of

ZO-1 and JAM-A to bacterial adherence sites<sup>[82]</sup>, and the mislocalization of ZO-1 to the basolateral membrane<sup>[91]</sup>. AGS cells that were incubated with Cag<sup>+</sup> *H. pylori* had severely damaged tight junctions and the presence of CagA resulted in cell scattering and a migratory phenotype consistent with the results in MDCK cells<sup>[89,92]</sup>. The same occurred in primary human cells that were cultured from antral mucosa<sup>[89]</sup>. In AGS cells, treatment with CagA<sup>+</sup> *H. pylori* also upregulated caudal type homeobox 2 (CDX2) and claudin 2 expression so it was concluded that CagA disrupts tight junctions by targeting claudin 2<sup>[92]</sup>. This is an interesting premise, however, because claudin 2 forms aqueous pores that are permeable to small cations<sup>[5]</sup> but does not cause tight junctions to form wide gaps and otherwise disassociate. CagA from *H. pylori* also localized with ZO-1 at tight junctions in T84 cells and over time, resulted in the enrichment of claudin 4<sup>[93]</sup>, suggesting that the transcriptional regulation of both claudin 2 and claudin 4 in *H. pylori* infection is *via* CagA. Work done jointly with MDCK and AGS cells were used to demonstrate that CagA specifically targets polarity-regulating kinase partitioning-defective 1b (Par1b)/MAP/microtubule affinity-regulated kinase 2 (MARK2) to disrupt apical tight junctions<sup>[94,95]</sup>, reduce TER<sup>[95]</sup>, and cause ZO-1 to disassemble from junctions<sup>[95]</sup>. It is interesting, however, that CagA<sup>+</sup> *H. pylori* did not affect tight junctions in Caco-2 intestinal cells<sup>[87]</sup> or in HGE-20 or MKN28 cultured human gastric epithelial cells<sup>[64,65]</sup>, in the human stomach, *in vivo*<sup>[54,65]</sup> or in mouse models of *H. pylori* infection *in vivo*<sup>[96,97]</sup>. To address the apparent differences *in vivo*, interesting experiments done recently in *Drosophila* identified numerous genetic modifiers of Cag-A induced epithelial disruption<sup>[98]</sup>. From a total of 10 genes whose expression significantly attenuates the effects of CagA were Lasp and chitinase 1<sup>[98]</sup>, both of which are highly expressed in gastric epithelial cells. Lasp-1 is highly expressed in parietal cells as a component of the actin cytoskeleton<sup>[99,100]</sup> and its activity is regulated by gastrin<sup>[101]</sup>, which is an important effector in *H. pylori* infection<sup>[102]</sup>. Chitinase 1 is also expressed in human and mouse stomach<sup>[103]</sup> but has a relatively unknown function. Overall these results suggest that CagA specifically targets components of the tight junction in addition to regulating the transcriptional program of gastric cells *in vitro*, but that the intact mucosa may express important modifiers that regulate CagA function to limit damage and preserve barrier function *in vivo*.

**VacA:** Disruption of the tight junction barrier by purified VacA from *H. pylori* was also done initially in MDCK cells<sup>[84]</sup>, demonstrating that TER declined in a pH-dependent manner when acid-activated toxin was used but that the decrement in TER did not occur by disrupting the integrity of tight junctions<sup>[84]</sup>. It was additionally shown that acid activation of VacA resulted in a pronounced increase in the permeability of mannitol and sucrose but not of inulin or HRP<sup>[84]</sup>.



**Table 2** Characteristics of human gastric cell lines that are used to study the role of *Helicobacter pylori* in tight junction dysfunction during infection

Cultured cell line	ZO-1, 2, or 3	Occludin	JAM's	Tricellulin	Cldns	Confluency	Ref.	Cldns not expressed
AGS	ZO-1				2, 4, 6, 7, 9	Confluent monolayer with no TER	[82,92,123-126]	11 <sup>[119]</sup>
BGC-823			JAM-A (low)		1, 18		[19,38,71]	
GES-1			JAM-A (high)				[19]	
HFE-145					11		[119]	
HGE-20	ZO-1				4	Confluent monolayer with TER-polarized	[65,80,81]	
HS-746T					1		[71]	
HSC-39					2		[111]	18 <sup>[34]</sup>
HSC-45				Yes			[16]	
HSC-57				Yes			[16]	
HSC-59				Yes			[16]	
KATOIII	ZO-1	Yes			1, 2, 4, 18		[38,71,72,111,126,127]	11 <sup>[119]</sup> , 18 <sup>[34]</sup>
MKN-1								4 <sup>[126]</sup> , 18 <sup>[34]</sup>
MKN-7	ZO-1	Yes		Yes	4		[16,127]	18 <sup>[34]</sup>
MKN-28	ZO-1	Yes	JAM-A		1, 3, 4, 7	Confluent monolayer with a TER	[64,71,72,126-128]	11 <sup>[119]</sup> , 18 <sup>[34]</sup>
MKN-45	ZO-1	Yes			1, 2, 3, 4, 18	Isolated cell clumps	[34,71,72,108,111,127]	
MKN-74				Yes	2, 4, 18		[16,34,37,38,111,126]	18 <sup>[108]</sup>
MUGC4					1		[72]	
NCI-N87	ZO-1				1, 4, 18	Confluent monolayer with a moderate TER	[38,66,71,126]	
NUGC-3								18 <sup>[108]</sup>
SIIA								11 <sup>[119]</sup>
SCG-7901			JAM-A		1, 18		[19,38,71]	
SNU-1								11 <sup>[119]</sup>
SNU-5					4		[126]	
SNU-216					4		[126]	
SNU-484								4 <sup>[126]</sup>
SNU-601					4		[126]	
SNU-620					4		[126]	
SNU-638								4 <sup>[126]</sup>
SNU-668								4 <sup>[126]</sup>
SNU-719					4		[126]	
TMK-1	ZO-1				1, 3, 4, 7, 12, 15, 18		[129]	18 <sup>[34]</sup>

Cldn: Claudin; ZO: Zonula occludens; TER: Transepithelial (electrical) resistance.

Moreover, VacA increased permeability to anions<sup>[84]</sup>. Pelicic *et al*<sup>[85]</sup> extended these findings to include *H. pylori*, and using the VacA<sup>+</sup>CagA<sup>+</sup> strain CCUG17874 and an isogenic VacA mutant demonstrated that VacA accounted for the entire decline in TER and increase in mannitol flux in MDCK cells. Overall these results suggested that VacA affects the tight junction pore pathway by increasing pore size and thus paracellular transport of small molecules (mannitol, 3.6 Å and sucrose 4.6 Å), but not the leak pathway, which would enhance the permeability of large molecules like inulin (11.5 Å) and HRP (24 Å)<sup>[11]</sup>. These results also suggested that changes in claudin expression occurred to increase the magnitude of flux through tight junctions in addition to changing permselectivity. In contrast to studies using MDCK cells, compelling results were obtained using the gastric NCI-N87 cell line; the TER was reduced with *H. pylori* and with each of the cytotoxin-associated isogenic mutants including VacA, CagA and urease subunit B (ureB),

suggesting that barrier dysfunction occurs in *H. pylori* infection independent of the associated virulence factors including VacA<sup>[66]</sup>. Caco-2 cells<sup>[87]</sup> and MKN28 cells<sup>[64]</sup> are additionally unaffected by VacA. VacA forms anion-selective channels, or pores, in cell or model membranes that share numerous properties with the host chloride, CLC, channels thus mimicking the characteristics of a host channel to conduct ions and perturb ion homeostasis in the stomach<sup>[104,105]</sup>. The VacA cytotoxin also promotes urea permeation in cultured MDCK, AGS, and Caco-2 cells<sup>[106]</sup> and was shown to enter cells, target mitochondria, and reduce mitochondrial membrane potential in a concentration-dependent manner<sup>[107]</sup>. Although it might be concluded that mitochondrial dysfunction would impact tight junction integrity by reducing ATP, urea permeation in the presence of VacA occurred by the transcellular, rather than paracellular, pathway and did not occur because of barrier dysfunction caused by damaging or otherwise reorganizing tight

junctions<sup>[106]</sup>. These studies further support the notion that VacA does not cause barrier dysfunction at tight junctions.

**Urease and ammonia:** In HGE-20 cell monolayers, luminal acid significantly increased TER and decreased paracellular permeability, which were affected by *H. pylori*, specifically in isogenic ureB<sup>-</sup> *H. pylori* mutants that produced considerable ammonia/ammonium<sup>[81]</sup>. This work suggested that *H. pylori* affects TER and permeability by neutralizing luminal acidity by the production of ammonia<sup>[66]</sup>. Although the claudin expression in this cell line is largely unknown (Table 1), it is tempting to speculate that claudin 18 is modulated by luminal acidity in HGE-20 cells. In general, extracellular acidity stimulates cell signaling pathways, including extracellular signal-regulated kinase (ERK) and protein kinase C activation, which were demonstrated to regulate the expression of claudin 18 in MKN-45 cells but not in MKN74 or NUGC3 cells, which do not express claudin 18<sup>[108]</sup>. Following apical acidification, claudin 18 expression increased TER and reduced paracellular permeability when overexpressed in MDCK cells<sup>[35]</sup> further supporting the idea that ureB<sup>+</sup> *H. pylori* and ammonia may reduce tight junction function by modulating the expression of claudin 18. Although MKN28 cells do not express claudin 18 (Table 1), this cell line also demonstrated a significant decrement in TER with (Vac<sup>+</sup>Cag<sup>+</sup>) *H. pylori* that required ureB and ammonia/ammonium. However, this study concluded that barrier disruption was due to the activation of myosin light chain kinase<sup>[64]</sup>. Although the TER in Caco-2 cells exposed to (Vac<sup>+</sup>Cag<sup>+</sup>) *H. pylori* was dependent on the ammonia/ammonium-induced processing of occludin to a low molecular weight form<sup>[87]</sup>, disruption of occludin does not cause barrier dysfunction in stomach, like it does in intestine<sup>[15]</sup>, so it is likely that results with occludin are not relevant to stomach cells, *in vivo*.

## CONCLUSION

In summary, tight junctions are configured slightly differently at the surface and in gastric glands but all claudin molecules are expressed in the mouse stomach with claudin 18 being the most prominent. While a comprehensive evaluation of claudin expression has not been done in the normal human stomach, human biopsy samples indicate that numerous claudins are expressed and that claudin 18 expression is also very high. These results suggest that stomach tight junctions are designed to be electrically tight and restrict cation permeability. Barrier dysfunction, including a reduction in TER and a significant increase in paracellular permeability, occurs *in vitro* and *in vivo* during *H. pylori* infection, consistent with a reduction in cation selectivity and an increase in the permeability of larger molecules due to significant changes in tight junction claudin expression and/or defects in tight

junction integrity. When evaluating the role of *H. pylori* virulence factors in tight junction dysfunction, the most consistent results occur with urease and ammonia, which are thought to cause cytoskeletal rearrangement at tight junctions. The changes in claudin expression in human *H. pylori*-induced GC are inconsistent, making it difficult to predict molecular mechanisms that regulate tight junction dysfunction in patients. On one hand, claudin 18 expression is generally attenuated while the expression of other cation-limiting claudins increases, perhaps to compensate for the lack of permselectivity and barrier tightness in the absence of claudin-18. For the most part, studies in human patients choose either a single or subset of tight junction proteins to survey, but this strategy provides an inadequate snapshot of the total set of abnormalities that occur in a given patient tumor. It is possible that genetic variation in virulence factors associated with *H. pylori*, host genetic factors, and constitutive levels of inflammation result in variable results in population studies of claudin expression and its relevance to long-term survival. Furthermore, studies are required to determine whether or not claudin molecules have other roles in gastric cells, besides their classical role at tight junctions, to facilitate cancer development. Genome sequencing and immunostaining with concomitant cell culture studies done in appropriate models may assist with future endeavors to sort-out the role of *H. pylori* in barrier defects during infection.

## REFERENCES

- 1 **Clark PM**, Dawany N, Dampier W, Byers SW, Pestell RG, Tozeren A. Bioinformatics analysis reveals transcriptome and microRNA signatures and drug repositioning targets for IBD and other autoimmune diseases. *Inflamm Bowel Dis* 2012; **18**: 2315-2333 [PMID: 22488912 DOI: 10.1002/ibd.22958]
- 2 **Lee SH**. Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases. *Intest Res* 2015; **13**: 11-18 [PMID: 25691839 DOI: 10.5217/ir.2015.13.1.11]
- 3 **Clayburgh DR**, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest* 2004; **84**: 282-291 [PMID: 14767487]
- 4 **Turner JR**, Buschmann MM, Romero-Calvo I, Sailer A, Shen L. The role of molecular remodeling in differential regulation of tight junction permeability. *Semin Cell Dev Biol* 2014; **36**: 204-212 [PMID: 25263012 DOI: 10.1016/j.semdb.2014.09.022]
- 5 **Krug SM**, Schulzke JD, Fromm M. Tight junction, selective permeability, and related diseases. *Semin Cell Dev Biol* 2014; **36**: 166-176 [PMID: 25220018 DOI: 10.1016/j.semdb.2014.09.002]
- 6 **Wroblewski LE**, Peek RM, Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. *Clin Microbiol Rev* 2010; **23**: 713-739 [PMID: 20930071 DOI: 10.1128/CMR.00011-10]
- 7 **Tsukita S**, Furuse M, Itoh M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol* 2001; **2**: 285-293 [PMID: 11283726]
- 8 **Suzuki H**, Tani K, Tamura A, Tsukita S, Fujiyoshi Y. Model for the architecture of claudin-based paracellular ion channels through tight junctions. *J Mol Biol* 2015; **427**: 291-297 [PMID: 25451028 DOI: 10.1016/j.jmb.2014.10.020]
- 9 **Van Itallie CM**, Anderson JM. Architecture of tight junctions and principles of molecular composition. *Semin Cell Dev Biol* 2014; **36**: 157-165 [PMID: 25171873 DOI: 10.1016/j.semdb.2014.08.011]

- 10 **Günzel D**, Yu AS. Claudins and the modulation of tight junction permeability. *Physiol Rev* 2013; **93**: 525-569 [PMID: 23589827 DOI: 10.1152/physrev.00019.2012]
- 11 **Anderson JM**, Van Itallie CM. Physiology and function of the tight junction. *Cold Spring Harb Perspect Biol* 2009; **1**: a002584 [PMID: 20066090 DOI: 10.1101/cshperspect.a002584]
- 12 **Claude P**, Goodenough DA. Fracture faces of zonulae occludentes from "tight" and "leaky" epithelia. *J Cell Biol* 1973; **58**: 390-400 [PMID: 4199658]
- 13 **Meyer RA**, McGinley D, Posalaky Z. The gastric mucosal barrier: structure of intercellular junctions in the dog. *J Ultrastruct Res* 1984; **86**: 192-201 [PMID: 6737566]
- 14 **Hayashi D**, Tamura A, Tanaka H, Yamazaki Y, Watanabe S, Suzuki K, Suzuki K, Sentani K, Yasui W, Rakugi H, Isaka Y, Tsukita S. Deficiency of claudin-18 causes paracellular H<sup>+</sup> leakage, up-regulation of interleukin-1 $\beta$ , and atrophic gastritis in mice. *Gastroenterology* 2012; **142**: 292-304 [PMID: 22079592 DOI: 10.1053/j.gastro.2011.10.040]
- 15 **Saitou M**, Furuse M, Sasaki H, Schulzke JD, Fromm M, Takano H, Noda T, Tsukita S. Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol Biol Cell* 2000; **11**: 4131-4142 [PMID: 11102513]
- 16 **Masuda R**, Semba S, Mizuuchi E, Yanagihara K, Yokozaki H. Negative regulation of the tight junction protein tricellulin by snail-induced epithelial-mesenchymal transition in gastric carcinoma cells. *Pathobiology* 2010; **77**: 106-113 [PMID: 20332670 DOI: 10.1159/000278293]
- 17 **Ohtani S**, Terashima M, Satoh J, Soeta N, Saze Z, Kashimura S, Ohsuka F, Hoshino Y, Kogure M, Gotoh M. Expression of tight-junction-associated proteins in human gastric cancer: downregulation of claudin-4 correlates with tumor aggressiveness and survival. *Gastric Cancer* 2009; **12**: 43-51 [PMID: 19390931 DOI: 10.1007/s10120-008-0497-0]
- 18 **Hajjari M**, Behmanesh M, Sadeghizadeh M, Zeinoddini M. Junctional adhesion molecules 2 and 3 may potentially be involved in progression of gastric adenocarcinoma tumors. *Med Oncol* 2013; **30**: 380 [PMID: 23277282 DOI: 10.1007/s12032-012-0380-z]
- 19 **Huang JY**, Xu YY, Sun Z, Wang ZN, Zhu Z, Song YX, Luo Y, Zhang X, Xu HM. Low junctional adhesion molecule A expression correlates with poor prognosis in gastric cancer. *J Surg Res* 2014; **192**: 494-502 [PMID: 25033702 DOI: 10.1016/j.jss.2014.06.025]
- 20 **Mariano C**, Silva SL, Pereira P, Fernandes A, Brites D, Brito MA. Evidence of tricellulin expression by immune cells, particularly microglia. *Biochem Biophys Res Commun* 2011; **409**: 799-802 [PMID: 21624353 DOI: 10.1016/j.bbrc.2011.05.093]
- 21 **Mineta K**, Yamamoto Y, Yamazaki Y, Tanaka H, Tada Y, Saito K, Tamura A, Igarashi M, Endo T, Takeuchi K, Tsukita S. Predicted expansion of the claudin multigene family. *FEBS Lett* 2011; **585**: 606-612 [PMID: 21276448 DOI: 10.1016/j.febslet.2011.01.028]
- 22 **Suzuki H**, Nishizawa T, Tani K, Yamazaki Y, Tamura A, Ishitani R, Dohmae N, Tsukita S, Nureki O, Fujiyoshi Y. Crystal structure of a claudin provides insight into the architecture of tight junctions. *Science* 2014; **344**: 304-307 [PMID: 24744376 DOI: 10.1126/science.1248571]
- 23 **Zhu JL**, Gao P, Wang ZN, Song YX, Li AL, Xu YY, Wang MX, Xu HM. Clinicopathological significance of claudin-4 in gastric carcinoma. *World J Surg Oncol* 2013; **11**: 150 [PMID: 23822740 DOI: 10.1186/1477-7819-11-150]
- 24 **Cunningham SC**, Kamangar F, Kim MP, Hammoud S, Haque R, Iacobuzio-Donahue CA, Maitra A, Ashfaq R, Hustinx S, Heitmiller RE, Choti MA, Lillemoe KD, Cameron JL, Yeo CJ, Schulick RD, Montgomery E. Claudin-4, mitogen-activated protein kinase kinase 4, and stratifin are markers of gastric adenocarcinoma precursor lesions. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 281-287 [PMID: 16492916]
- 25 **Wang H**, Yang X. The expression patterns of tight junction protein claudin-1, -3, and -4 in human gastric neoplasms and adjacent non-neoplastic tissues. *Int J Clin Exp Pathol* 2015; **8**: 881-887 [PMID: 25755790]
- 26 **Lee LY**, Wu CM, Wang CC, Yu JS, Liang Y, Huang KH, Lo CH, Hwang TL. Expression of matrix metalloproteinases MMP-2 and MMP-9 in gastric cancer and their relation to claudin-4 expression. *Histol Histopathol* 2008; **23**: 515-521 [PMID: 18283635]
- 27 **Lin Z**, Zhang X, Liu Z, Liu Q, Wang L, Lu Y, Liu Y, Wang M, Yang M, Jin X, Quan C. The distinct expression patterns of claudin-2, -6, and -11 between human gastric neoplasms and adjacent non-neoplastic tissues. *Diagn Pathol* 2013; **8**: 133 [PMID: 23919729 DOI: 10.1186/1746-1596-8-133]
- 28 **Psáder R**, Jakab C, Máthé A, Balka G, Pápa K, Sterczler A. Expression of claudins in the normal canine gastric mucosa. *Acta Vet Hung* 2014; **62**: 13-21 [PMID: 24334088 DOI: 10.1556/AVet.2013.053]
- 29 **Rahner C**, Mitic LL, Anderson JM. Heterogeneity in expression and subcellular localization of claudins 2, 3, 4, and 5 in the rat liver, pancreas, and gut. *Gastroenterology* 2001; **120**: 411-422 [PMID: 11159882]
- 30 **Troy TC**, Arabzadeh A, Yerlikaya S, Turksen K. Claudin immunolocalization in neonatal mouse epithelial tissues. *Cell Tissue Res* 2007; **330**: 381-388 [PMID: 17828607]
- 31 **Inai T**, Sengoku A, Guan X, Hirose E, Iida H, Shibata Y. Heterogeneity in expression and subcellular localization of tight junction proteins, claudin-10 and -15, examined by RT-PCR and immunofluorescence microscopy. *Arch Histol Cytol* 2005; **68**: 349-360 [PMID: 16505581]
- 32 **Sakamoto H**, Mutoh H, Sugano K. Expression of Claudin-2 in intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach. *Scand J Gastroenterol* 2010; **45**: 1273-1280 [PMID: 20602571 DOI: 10.3109/00365521.2010.501522]
- 33 **Niimi T**, Nagashima K, Ward JM, Minoo P, Zimonjic DB, Popescu NC, Kimura S. claudin-18, a novel downstream target gene for the T/EBP/NKX2.1 homeodomain transcription factor, encodes lung- and stomach-specific isoforms through alternative splicing. *Mol Cell Biol* 2001; **21**: 7380-7390 [PMID: 11585919]
- 34 **Sanada Y**, Oue N, Mitani Y, Yoshida K, Nakayama H, Yasui W. Down-regulation of the claudin-18 gene, identified through serial analysis of gene expression data analysis, in gastric cancer with an intestinal phenotype. *J Pathol* 2006; **208**: 633-642 [PMID: 16435283]
- 35 **Jovov B**, Van Itallie CM, Shaheen NJ, Carson JL, Gambling TM, Anderson JM, Orlando RC. Claudin-18: a dominant tight junction protein in Barrett's esophagus and likely contributor to its acid resistance. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G1106-G1113 [PMID: 17932229]
- 36 **Jun KH**, Kim JH, Jung JH, Choi HJ, Chin HM. Expression of claudin-7 and loss of claudin-18 correlate with poor prognosis in gastric cancer. *Int J Surg* 2014; **12**: 156-162 [PMID: 24333468 DOI: 10.1016/j.ijsu.2013.11.022]
- 37 **Oshima T**, Shan J, Okugawa T, Chen X, Hori K, Tomita T, Fukui H, Watari J, Miwa H. Down-regulation of claudin-18 is associated with the proliferative and invasive potential of gastric cancer at the invasive front. *PLoS One* 2013; **8**: e74757 [PMID: 24073219 DOI: 10.1371/journal.pone.0074757]
- 38 **Zhang SJ**, Feng JF, Wang L, Guo W, Du YW, Ming L, Zhao GQ. miR-1303 targets claudin-18 gene to modulate proliferation and invasion of gastric cancer cells. *Dig Dis Sci* 2014; **59**: 1754-1763 [PMID: 24647998 DOI: 10.1007/s10620-014-3107-5]
- 39 **Fox JG**, Lee A. The role of Helicobacter species in newly recognized gastrointestinal tract diseases of animals. *Lab Anim Sci* 1997; **47**: 222-255 [PMID: 9241625]
- 40 **Sachs G**, Weeks DL, Melchers K, Scott DR. The gastric biology of Helicobacter pylori. *Annu Rev Physiol* 2003; **65**: 349-369 [PMID: 12471160]
- 41 **Sachs G**, Kraut JA, Wen Y, Feng J, Scott DR. Urea transport in bacteria: acid acclimation by gastric Helicobacter spp. *J Membr Biol* 2006; **212**: 71-82 [PMID: 17264989]
- 42 **Mobley HL**, Hu LT, Foxal PA. Helicobacter pylori urease: properties and role in pathogenesis. *Scand J Gastroenterol Suppl* 1991; **187**: 39-46 [PMID: 1775923]
- 43 **Moran AP**. Lipopolysaccharide in bacterial chronic infection: insights from Helicobacter pylori lipopolysaccharide and lipid A.

- Int J Med Microbiol* 2007; **297**: 307-319 [PMID: 17467335]
- 44 **Hanson PJ**, Moran AP, Butler K. Paracellular permeability is increased by basal lipopolysaccharide in a primary culture of colonic epithelial cells; an effect prevented by an activator of Toll-like receptor-2. *Innate Immun* 2011; **17**: 269-282 [PMID: 20472611 DOI: 10.1177/1753425910367813]
- 45 **World Health Organization**. Infection with *Helicobacter pylori*. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 177-240 [PMID: 7715070]
- 46 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
- 47 **Colquhoun A**, Arnold M, Ferlay J, Goodman KJ, Forman D, Soerjomataram I. Global patterns of cardia and non-cardia gastric cancer incidence in 2012. *Gut* 2015; Epub ahead of print [PMID: 25748648 DOI: 10.1136/gutjnl-2014-308915]
- 48 **Correa P**. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; **52**: 6735-6740 [PMID: 1458460]
- 49 **Correa P**, Houghton J. Carcinogenesis of *Helicobacter pylori*. *Gastroenterology* 2007; **133**: 659-672 [PMID: 17681184]
- 50 **Lu B**, Li M. *Helicobacter pylori* eradication for preventing gastric cancer. *World J Gastroenterol* 2014; **20**: 5660-5665 [PMID: 24914325 DOI: 10.3748/wjg.v20.i19.5660]
- 51 **Choi J**, Kim SG, Yoon H, Im JP, Kim JS, Kim WH, Jung HC. Eradication of *Helicobacter pylori* after endoscopic resection of gastric tumors does not reduce incidence of metachronous gastric carcinoma. *Clin Gastroenterol Hepatol* 2014; **12**: 793-800.e1 [PMID: 24100112 DOI: 10.1016/j.cgh.2013.09.057]
- 52 **Bode G**, Malfertheiner P, Ditschuneit H. Pathogenetic implications of ultrastructural findings in *Campylobacter pylori* related gastroduodenal disease. *Scand J Gastroenterol Suppl* 1988; **142**: 25-39 [PMID: 3166531]
- 53 **Chen XG**, Correa P, Offerhaus J, Rodriguez E, Janney F, Hoffmann E, Fox J, Hunter F, Diavolitsis S. Ultrastructure of the gastric mucosa harboring *Campylobacter*-like organisms. *Am J Clin Pathol* 1986; **86**: 575-582 [PMID: 2430450]
- 54 **Necchi V**, Candusso ME, Tava F, Luinetti O, Ventura U, Fiocca R, Ricci V, Solcia E. Intracellular, intercellular, and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by *Helicobacter pylori*. *Gastroenterology* 2007; **132**: 1009-1023 [PMID: 17383424]
- 55 **Noach LA**, Rolf TM, Tytgat GN. Electron microscopic study of association between *Helicobacter pylori* and gastric and duodenal mucosa. *J Clin Pathol* 1994; **47**: 699-704 [PMID: 7962619]
- 56 **Fox JG**, Wang TC. Inflammation, atrophy, and gastric cancer. *J Clin Invest* 2007; **117**: 60-69 [PMID: 17200707]
- 57 **Hopwood D**, Milne G, Penston J. Leakiness of gastric superficial and foveolar cells. A quantitative electron microscopic study using tannic acid. *J Pathol* 1991; **165**: 119-124 [PMID: 1744797]
- 58 **Borch K**, Sjöstedt C, Hannestad U, Söderholm JD, Franzén L, Mårdh S. Asymptomatic *Helicobacter pylori* gastritis is associated with increased sucrose permeability. *Dig Dis Sci* 1998; **43**: 749-753 [PMID: 9558030]
- 59 **Matysiak-Budnik T**, Coffin B, Lavergne-Slove A, Sabate JM, Mégraud F, Heyman M. *Helicobacter pylori* increases the epithelial permeability to a food antigen in human gastric biopsies. *Am J Gastroenterol* 2004; **99**: 225-232 [PMID: 15046209]
- 60 **Fukuda Y**, Bamba H, Okui M, Tamura K, Tanida N, Satomi M, Shimoyama T, Nishigami T. *Helicobacter pylori* infection increases mucosal permeability of the stomach and intestine. *Digestion* 2001; **63 Suppl 1**: 93-96 [PMID: 11173917]
- 61 **Suzuki K**, Kokai Y, Sawada N, Takakuwa R, Kuwahara K, Isogai E, Isogai H, Mori M. S51 *Helicobacter pylori* disrupts the paracellular barrier of the gastric mucosa and leads to neutrophilic gastritis in mice. *Virchows Arch* 2002; **440**: 318-324 [PMID: 11889604]
- 62 **Fedwick JP**, Lapointe TK, Meddings JB, Sherman PM, Buret AG. *Helicobacter pylori* activates myosin light-chain kinase to disrupt claudin-4 and claudin-5 and increase epithelial permeability. *Infect Immun* 2005; **73**: 7844-7852 [PMID: 16299274]
- 63 **Matysiak-Budnik T**, Hashimoto K, Heyman M, de Mascarel A, Desjeux JF, Mégraud F. Antral gastric permeability to antigens in mice is altered by infection with *Helicobacter felis*. *Eur J Gastroenterol Hepatol* 1999; **11**: 1371-1377 [PMID: 10654797]
- 64 **Wroblewski LE**, Shen L, Ogden S, Romero-Gallo J, Lapiere LA, Israel DA, Turner JR, Peek RM. *Helicobacter pylori* dysregulation of gastric epithelial tight junctions by urease-mediated myosin II activation. *Gastroenterology* 2009; **136**: 236-246 [PMID: 18996125 DOI: 10.1053/j.gastro.2008.10.011]
- 65 **Lapointe TK**, O'Connor PM, Jones NL, Menard D, Buret AG. Interleukin-1 receptor phosphorylation activates Rho kinase to disrupt human gastric tight junctional claudin-4 during *Helicobacter pylori* infection. *Cell Microbiol* 2010; **12**: 692-703 [PMID: 20070312 DOI: 10.1111/j.1462-5822.2010.01429.x]
- 66 **Fiorentino M**, Ding H, Blanchard TG, Czinn SJ, Szein MB, Fasano A. *Helicobacter pylori*-induced disruption of monolayer permeability and proinflammatory cytokine secretion in polarized human gastric epithelial cells. *Infect Immun* 2013; **81**: 876-883 [PMID: 23297384 DOI: 10.1128/IAI.01406-12]
- 67 **Eftang LL**, Esbensen Y, Tannæs TM, Blom GP, Bukholm IR, Bukholm G. Up-regulation of CLDN1 in gastric cancer is correlated with reduced survival. *BMC Cancer* 2013; **13**: 586 [PMID: 24321518 DOI: 10.1186/1471-2407-13-586]
- 68 **Jung H**, Jun KH, Jung JH, Chin HM, Park WB. The expression of claudin-1, claudin-2, claudin-3, and claudin-4 in gastric cancer tissue. *J Surg Res* 2011; **167**: e185-e191 [PMID: 20462599 DOI: 10.1016/j.jss.2010.02.010]
- 69 **Soini Y**, Tammola S, Helin H, Martikainen P. Claudins 1, 3, 4 and 5 in gastric carcinoma, loss of claudin expression associates with the diffuse subtype. *Virchows Arch* 2006; **448**: 52-58 [PMID: 16220299]
- 70 **Wu YL**, Zhang S, Wang GR, Chen YP. Expression transformation of claudin-1 in the process of gastric adenocarcinoma invasion. *World J Gastroenterol* 2008; **14**: 4943-4948 [PMID: 18756604]
- 71 **Huang J**, Zhang L, He C, Qu Y, Li J, Zhang J, Du T, Chen X, Yu Y, Liu B, Zhu Z. Claudin-1 enhances tumor proliferation and metastasis by regulating cell anoikis in gastric cancer. *Oncotarget* 2015; **6**: 1652-1665 [PMID: 25544763]
- 72 **Shiozaki A**, Shimizu H, Ichikawa D, Konishi H, Komatsu S, Kubota T, Fujiwara H, Okamoto K, Iitaka D, Nakashima S, Nako Y, Liu M, Otsuji E. Claudin 1 mediates tumor necrosis factor alpha-induced cell migration in human gastric cancer cells. *World J Gastroenterol* 2014; **20**: 17863-17876 [PMID: 25548484 DOI: 10.3748/wjg.v20.i47.17863]
- 73 **Chang TL**, Ito K, Ko TK, Liu Q, Salto-Tellez M, Yeoh KG, Fukamachi H, Ito Y. Claudin-1 has tumor suppressive activity and is a direct target of RUNX3 in gastric epithelial cells. *Gastroenterology* 2010; **138**: 255-65.e1-3 [PMID: 19706291 DOI: 10.1053/j.gastro.2009.08.044]
- 74 **Resnick MB**, Gavilanez M, Newton E, Konkin T, Bhattacharya B, Britt DE, Sabo E, Moss SF. Claudin expression in gastric adenocarcinomas: a tissue microarray study with prognostic correlation. *Hum Pathol* 2005; **36**: 886-892 [PMID: 16112005]
- 75 **Iravani O**, Tay BW, Chua PJ, Yip GW, Bay BH. Claudins and gastric carcinogenesis. *Exp Biol Med (Maywood)* 2013; **238**: 344-349 [PMID: 23759999 DOI: 10.1177/1535370213477981]
- 76 **Takaishi S**, Wang TC. Gene expression profiling in a mouse model of *Helicobacter*-induced gastric cancer. *Cancer Sci* 2007; **98**: 284-293 [PMID: 17270017]
- 77 **Tatum R**, Zhang Y, Salleng K, Lu Z, Lin JJ, Lu Q, Jeansonne BG, Ding L, Chen YH. Renal salt wasting and chronic dehydration in claudin-7-deficient mice. *Am J Physiol Renal Physiol* 2010; **298**: F24-F34 [PMID: 19759267 DOI: 10.1152/ajprenal.00450.2009]
- 78 **Nübel T**, Preobraschenski J, Tuncay H, Weiss T, Kuhn S, Ladwein M, Langbein L, Zöller M. Claudin-7 regulates EpCAM-mediated functions in tumor progression. *Mol Cancer Res* 2009; **7**: 285-299 [PMID: 19276185 DOI: 10.1158/1541-7786.MCR-08-0200]



- 79 **Wenqi D**, Li W, Shanshan C, Bei C, Yafei Z, Feihu B, Jie L, Daiming F. EpCAM is overexpressed in gastric cancer and its downregulation suppresses proliferation of gastric cancer. *J Cancer Res Clin Oncol* 2009; **135**: 1277-1285 [PMID: 19294417 DOI: 10.1007/s00432-009-0569-5]
- 80 **Chailier P**, Ménard D. Establishment of human gastric epithelial (HGE) cell lines exhibiting barrier function, progenitor, and prezymogenic characteristics. *J Cell Physiol* 2005; **202**: 263-274 [PMID: 15389599]
- 81 **Marcus EA**, Vagin O, Tokhtaeva E, Sachs G, Scott DR. Helicobacter pylori impedes acid-induced tightening of gastric epithelial junctions. *Am J Physiol Gastrointest Liver Physiol* 2013; **305**: G731-G739 [PMID: 23989011 DOI: 10.1152/ajpgi.00209.2013]
- 82 **Amieva MR**, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S. Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA. *Science* 2003; **300**: 1430-1434 [PMID: 12775840]
- 83 **Xin S**, Huixin C, Benchang S, Aiping B, Jinhui W, Xiaoyan L, Yu WB, Minhu C. Expression of Cdx2 and claudin-2 in the multistage tissue of gastric carcinogenesis. *Oncology* 2007; **73**: 357-365 [PMID: 18500171 DOI: 10.1159/000135351]
- 84 **Papini E**, Satin B, Norais N, de Bernard M, Telford JL, Rappuoli R, Montecucco C. Selective increase of the permeability of polarized epithelial cell monolayers by Helicobacter pylori vacuolating toxin. *J Clin Invest* 1998; **102**: 813-820 [PMID: 9710450]
- 85 **Pelicic V**, Reytrat JM, Sartori L, Pagliaccia C, Rappuoli R, Telford JL, Montecucco C, Papini E. Helicobacter pylori VacA cytotoxin associated with the bacteria increases epithelial permeability independently of its vacuolating activity. *Microbiology* 1999; **145** (Pt 8): 2043-2050 [PMID: 10463170]
- 86 **Terrés AM**, Pajares JM, Hopkins AM, Murphy A, Moran A, Baird AW, Kelleher D. Helicobacter pylori disrupts epithelial barrier function in a process inhibited by protein kinase C activators. *Infect Immun* 1998; **66**: 2943-2950 [PMID: 9596771]
- 87 **Lytton SD**, Fischer W, Nagel W, Haas R, Beck FX. Production of ammonium by Helicobacter pylori mediates occludin processing and disruption of tight junctions in Caco-2 cells. *Microbiology* 2005; **151**: 3267-3276 [PMID: 16207910]
- 88 **Wroblewski LE**, Piazuolo MB, Chaturvedi R, Schumacher M, Aihara E, Feng R, Noto JM, Delgado A, Israel DA, Zavros Y, Montrose MH, Shroyer N, Correa P, Wilson KT, Peek RM. Helicobacter pylori targets cancer-associated apical-junctional constituents in gastroids and gastric epithelial cells. *Gut* 2015; **64**: 720-730 [PMID: 25123931 DOI: 10.1136/gutjnl-2014-307650]
- 89 **Lai YP**, Yang JC, Lin TZ, Lin JT, Wang JT. Helicobacter pylori infection and CagA protein translocation in human primary gastric epithelial cell culture. *Helicobacter* 2006; **11**: 451-459 [PMID: 16961808]
- 90 **Krueger S**, Hundertmark T, Kuester D, Kalinski T, Peitz U, Roessner A. Helicobacter pylori alters the distribution of ZO-1 and p120ctn in primary human gastric epithelial cells. *Pathol Res Pract* 2007; **203**: 433-444 [PMID: 17509776]
- 91 **Bagnoli F**, Buti L, Tompkins L, Covacci A, Amieva MR. Helicobacter pylori CagA induces a transition from polarized to invasive phenotypes in MDCK cells. *Proc Natl Acad Sci USA* 2005; **102**: 16339-16344 [PMID: 16258069]
- 92 **Song X**, Chen HX, Wang XY, Deng XY, Xi YX, He Q, Peng TL, Chen J, Chen W, Wong BC, Chen MH. H. pylori-encoded CagA disrupts tight junctions and induces invasiveness of AGS gastric carcinoma cells via Cdx2-dependent targeting of Claudin-2. *Cell Immunol* 2013; **286**: 22-30 [PMID: 24287273 DOI: 10.1016/j.cellimm.2013.10.008]
- 93 **El-Etr SH**, Mueller A, Tompkins LS, Falkow S, Merrell DS. Phosphorylation-independent effects of CagA during interaction between Helicobacter pylori and T84 polarized monolayers. *J Infect Dis* 2004; **190**: 1516-1523 [PMID: 15378446]
- 94 **Zeaier Z**, Cohen D, Müsch A, Bagnoli F, Covacci A, Stein M. Analysis of detergent-resistant membranes of Helicobacter pylori infected gastric adenocarcinoma cells reveals a role for MARK2/Par1b in CagA-mediated disruption of cellular polarity. *Cell Microbiol* 2008; **10**: 781-794 [PMID: 18005242]
- 95 **Saadat I**, Higashi H, Obuse C, Umeda M, Murata-Kamiya N, Saito Y, Lu H, Ohnishi N, Azuma T, Suzuki A, Ohno S, Hatakeyama M. Helicobacter pylori CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. *Nature* 2007; **447**: 330-333 [PMID: 17507984]
- 96 **Hagen SJ**, Yang DX, Tashima K, Taylor NS, Fox JG. Epithelial cell expression of BCL-2 family proteins predicts mechanisms that regulate Helicobacter pylori-induced pathology in the mouse stomach. *Lab Invest* 2008; **88**: 1227-1244 [PMID: 18779780 DOI: 10.1038/labinvest.2008.84]
- 97 **Hagen SJ**, Ohtani M, Zhou JR, Taylor NS, Rickman BH, Blackburn GL, Fox JG. Inflammation and foveolar hyperplasia are reduced by supplemental dietary glutamine during Helicobacter pylori infection in mice. *J Nutr* 2009; **139**: 912-918 [PMID: 19261732 DOI: 10.3945/jn.108.097790]
- 98 **Reid DW**, Muyskens JB, Neal JT, Gaddini GW, Cho LY, Wandler AM, Botham CM, Guillemin K. Identification of genetic modifiers of CagA-induced epithelial disruption in Drosophila. *Front Cell Infect Microbiol* 2012; **2**: 24 [PMID: 22919616 DOI: 10.3389/fcimb.2012.00024]
- 99 **Chew CS**, Parente JA, Chen X, Chaponnier C, Cameron RS. The LIM and SH3 domain-containing protein, lasp-1, may link the cAMP signaling pathway with dynamic membrane restructuring activities in ion transporting epithelia. *J Cell Sci* 2000; **113** (Pt 11): 2035-2045 [PMID: 10806114]
- 100 **Chew CS**, Parente JA, Zhou C, Baranco E, Chen X. Lasp-1 is a regulated phosphoprotein within the cAMP signaling pathway in the gastric parietal cell. *Am J Physiol* 1998; **275**: C56-C67 [PMID: 9688835]
- 101 **Jain RN**, Brunkan CS, Chew CS, Samuelson LC. Gene expression profiling of gastrin target genes in parietal cells. *Physiol Genomics* 2006; **24**: 124-132 [PMID: 16278279]
- 102 **Wang TC**, Dangler CA, Chen D, Goldenring JR, Koh T, Raychowdhury R, Coffey RJ, Ito S, Varro A, Dockray GJ, Fox JG. Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. *Gastroenterology* 2000; **118**: 36-47 [PMID: 10611152]
- 103 **Ohno M**, Togashi Y, Tsuda K, Okawa K, Kamaya M, Sakaguchi M, Sugahara Y, Oyama F. Quantification of Chitinase mRNA Levels in Human and Mouse Tissues by Real-Time PCR: Species-Specific Expression of Acidic Mammalian Chitinase in Stomach Tissues. *PLoS One* 2013; **8**: e67399 [PMID: 23826286]
- 104 **Tombola F**, Carlesso C, Szabò I, de Bernard M, Reytrat JM, Telford JL, Rappuoli R, Montecucco C, Papini E, Zoratti M. Helicobacter pylori vacuolating toxin forms anion-selective channels in planar lipid bilayers: possible implications for the mechanism of cellular vacuolation. *Biophys J* 1999; **76**: 1401-1409 [PMID: 10049322]
- 105 **Czajkowsky DM**, Iwamoto H, Szabo G, Cover TL, Shao Z. Mimicry of a host anion channel by a Helicobacter pylori pore-forming toxin. *Biophys J* 2005; **89**: 3093-3101 [PMID: 16100263]
- 106 **Tombola F**, Morbiato L, Del Giudice G, Rappuoli R, Zoratti M, Papini E. The Helicobacter pylori VacA toxin is a urea permease that promotes urea diffusion across epithelia. *J Clin Invest* 2001; **108**: 929-937 [PMID: 11560962]
- 107 **Willhite DC**, Blanke SR. Helicobacter pylori vacuolating cytotoxin enters cells, localizes to the mitochondria, and induces mitochondrial membrane permeability changes correlated to toxin channel activity. *Cell Microbiol* 2004; **6**: 143-154 [PMID: 14706100]
- 108 **Yano K**, Imaeda T, Niimi T. Transcriptional activation of the human claudin-18 gene promoter through two AP-1 motifs in PMA-stimulated MKN45 gastric cancer cells. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G336-G343 [PMID: 18032479]
- 109 **Karam SM**. A focus on parietal cells as a renewing cell population. *World J Gastroenterol* 2010; **16**: 538-546 [PMID: 20128020]
- 110 **Hewitt KJ**, Agarwal R, Morin PJ. The claudin gene family: expression in normal and neoplastic tissues. *BMC Cancer* 2006; **6**:

- 186 [PMID: 16836752]
- 111 **Aung PP**, Mitani Y, Sanada Y, Nakayama H, Matsusaki K, Yasui W. Differential expression of claudin-2 in normal human tissues and gastrointestinal carcinomas. *Virchows Arch* 2006; **448**: 428-434 [PMID: 16328347]
- 112 **Kamata I**, Ishikawa Y, Akishima-Fukasawa Y, Ito K, Akasaka Y, Uzuki M, Fujimoto A, Morita H, Tamai S, Maehara T, Ogata K, Shimokawa R, Igarashi Y, Miki K, Ishii T. Significance of lymphatic invasion and cancer invasion-related proteins on lymph node metastasis in gastric cancer. *J Gastroenterol Hepatol* 2009; **24**: 1527-1533 [PMID: 19383080 DOI: 10.1111/j.1440-1746.2009.05810.x]
- 113 **Matsuda Y**, Semba S, Ueda J, Fuku T, Hasuo T, Chiba H, Sawada N, Kuroda Y, Yokozaki H. Gastric and intestinal claudin expression at the invasive front of gastric carcinoma. *Cancer Sci* 2007; **98**: 1014-1019 [PMID: 17459057]
- 114 **Semba S**, Hasuo T, Satake S, Nakayama F, Yokozaki H. Prognostic significance of intestinal claudins in high-risk synchronous and metachronous multiple gastric epithelial neoplasias after initial endoscopic submucosal dissection. *Pathol Int* 2008; **58**: 371-377 [PMID: 18477216 DOI: 10.1111/j.1440-1827.2008.02238.x]
- 115 **Okugawa T**, Oshima T, Chen X, Hori K, Tomita T, Fukui H, Watari J, Matsumoto T, Miwa H. Down-regulation of claudin-3 is associated with proliferative potential in early gastric cancers. *Dig Dis Sci* 2012; **57**: 1562-1567 [PMID: 22290341 DOI: 10.1007/s10620-012-2043-5]
- 116 **Imura J**, Hayashi S, Ichikawa K, Miwa S, Nakajima T, Nomoto K, Tsuneyama K, Nogami T, Saitoh H, Fujimori T. Malignant transformation of hyperplastic gastric polyps: An immunohistochemical and pathological study of the changes of neoplastic phenotype. *Oncol Lett* 2014; **7**: 1459-1463 [PMID: 24765156]
- 117 **Kuo WL**, Lee LY, Wu CM, Wang CC, Yu JS, Liang Y, Lo CH, Huang KH, Hwang TL. Differential expression of claudin-4 between intestinal and diffuse-type gastric cancer. *Oncol Rep* 2006; **16**: 729-734 [PMID: 16969486]
- 118 **Gao M**, Li W, Wang H, Wang G. The distinct expression patterns of claudin-10, -14, -17 and E-cadherin between adjacent non-neoplastic tissues and gastric cancer tissues. *Diagn Pathol* 2013; **8**: 205 [PMID: 24325792 DOI: 10.1186/1746-1596-8-205]
- 119 **Agarwal R**, Mori Y, Cheng Y, Jin Z, Olaru AV, Hamilton JP, David S, Selaru FM, Yang J, Abraham JM, Montgomery E, Morin PJ, Meltzer SJ. Silencing of claudin-11 is associated with increased invasiveness of gastric cancer cells. *PLoS One* 2009; **4**: e8002 [PMID: 19956721 DOI: 10.1371/journal.pone.0008002]
- 120 **Türeci O**, Koslowski M, Helftenbein G, Castle J, Rohde C, Dhaene K, Seitz G, Sahin U. Claudin-18 gene structure, regulation, and expression is evolutionary conserved in mammals. *Gene* 2011; **481**: 83-92 [PMID: 21571049 DOI: 10.1016/j.gene.2011.04.007]
- 121 **Katoh M**, Katoh M. CLDN23 gene, frequently down-regulated in intestinal-type gastric cancer, is a novel member of CLAUDIN gene family. *Int J Mol Med* 2003; **11**: 683-689 [PMID: 12736707]
- 122 **Hasegawa S**, Furukawa Y, Li M, Satoh S, Kato T, Watanabe T, Katagiri T, Tsunoda T, Yamaoka Y, Nakamura Y. Genome-wide analysis of gene expression in intestinal-type gastric cancers using a complementary DNA microarray representing 23,040 genes. *Cancer Res* 2002; **62**: 7012-7017 [PMID: 12460921]
- 123 **Zavala-Zendejas VE**, Torres-Martinez AC, Salas-Morales B, Fortoul TI, Montañó LF, Rendon-Huerta EP. Claudin-6, 7, or 9 overexpression in the human gastric adenocarcinoma cell line AGS increases its invasiveness, migration, and proliferation rate. *Cancer Invest* 2011; **29**: 1-11 [PMID: 20874001 DOI: 10.3109/07357907.2010.512594]
- 124 **Hwang TL**, Changchien TT, Wang CC, Wu CM. Claudin-4 expression in gastric cancer cells enhances the invasion and is associated with the increased level of matrix metalloproteinase-2 and -9 expression. *Oncol Lett* 2014; **8**: 1367-1371 [PMID: 25120725]
- 125 **Mima S**, Tsutsumi S, Ushijima H, Takeda M, Fukuda I, Yokomizo K, Suzuki K, Sano K, Nakanishi T, Tomisato W, Tsuchiya T, Mizushima T. Induction of claudin-4 by nonsteroidal anti-inflammatory drugs and its contribution to their chemopreventive effect. *Cancer Res* 2005; **65**: 1868-1876 [PMID: 15753385]
- 126 **Kwon MJ**, Kim SH, Jeong HM, Jung HS, Kim SS, Lee JE, Gye MC, Erkin OC, Koh SS, Choi YL, Park CK, Shin YK. Claudin-4 overexpression is associated with epigenetic derepression in gastric carcinoma. *Lab Invest* 2011; **91**: 1652-1667 [PMID: 21844869 DOI: 10.1038/labinvest.2011.117]
- 127 **Atsumi T**, Kato K, Uno K, Iijima K, Koike T, Imatani A, Ohara S, Shimosegawa T. Pathophysiological role of the activation of p38 mitogen-activated protein kinases in poorly differentiated gastric cancer. *Pathol Int* 2007; **57**: 635-644 [PMID: 17803652]
- 128 **Hashimoto K**, Oshima T, Tomita T, Kim Y, Matsumoto T, Joh T, Miwa H. Oxidative stress induces gastric epithelial permeability through claudin-3. *Biochem Biophys Res Commun* 2008; **376**: 154-157 [PMID: 18774778 DOI: 10.1016/j.bbrc.2008.08.140]
- 129 **Satake S**, Semba S, Matsuda Y, Usami Y, Chiba H, Sawada N, Kasuga M, Yokozaki H. Cdx2 transcription factor regulates claudin-3 and claudin-4 expression during intestinal differentiation of gastric carcinoma. *Pathol Int* 2008; **58**: 156-163 [PMID: 18251778 DOI: 10.1111/j.1440-1827.2007.02204.x]

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