

Claudins in intestines

Distribution and functional significance in health and diseases

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Intestines are organs that not only digest food and absorb nutrients, but also provide a defense barrier against pathogens and noxious agents ingested. Tight junctions (TJs) are the most apical component of the junctional complex, providing one form of cell-cell adhesion in enterocytes and playing a critical role in regulating paracellular barrier permeability. Alteration of TJs leads to a number of pathophysiological diseases causing malabsorption of nutrition and intestinal structure disruption, which may even contribute to systemic organ failure. Claudins are the major structural and functional components of TJs with at least 24 members in mammals. Claudins have distinct charge-selectivity, either by tightening the paracellular pathway or functioning as paracellular channels, regulating ions and small molecules passing through the paracellular pathway. In this review, we have discussed the functions of claudin family members, their distribution and localization in the intestinal tract of mammals, their alterations in intestine-related diseases and chemicals/agents that regulate the expression and localization of claudins as well as the intestinal permeability, which provide a therapeutic view for treating intestinal diseases.

Claudin Distributions and Functions in Intestines

Intestinal epithelial integrity is vital for nutrition absorption and host defense against pathogens. The gastrointestinal (GI) tract can be divided into the small intestine that includes duodenum, jejunum and ileum and the large intestine that contains cecum and colon. Both intestines share a general structure with four layers: mucosa, submucosa, muscularis externa and serosa. Intestinal epithelium (enterocytes) is in the mucosa layer. Tight junctions (TJs) are the most apical structure present in the junctional complex between the epithelial cells. They form a circumferential belt around the epithelial sheet, separating the cell membrane

into apical (the side toward the lumen) and basolateral (the side facing the extracellular matrix) domains. The permeability of TJs varies in different segments of intestines. Well-formed TJs are characterized by high transepithelial electrical resistance (TER) and low solute permeability. The permeability of TJs can be determined by measuring the paracellular fluxes of ions and small molecules such as ³H-mannitol and FITC-dextran. The permeability of TJs changes under different physiological and pathological conditions.

There are three main families of TJ proteins: the claudin family, the occludin family and the IgG-like family of junctional adhesion molecules (JAMs).¹ Claudins are the main determinants of barrier properties of the TJs. Up to now, at least 24 members of the claudin family have been identified. Claudins are transmembrane proteins containing two extracellular domains and one intracellular domain with N- and C-termini facing the cytoplasm.² Many claudins have distinct charge-selectivity. Some claudins act by plugging the paracellular pathway while others function as paracellular channels. For example, claudin-2 and -15 are cation-selective while claudin-17 is anion-selective.³ The incorporation and association of occludin, claudins and JAMs into TJ strands require local clustering of scaffolding proteins, an important group of which is ZO proteins, such as ZO-1, ZO-2 and ZO-3.⁴

Differential expression patterns of claudin members in the GI tract are likely to contribute to local diversity of TER and paracellular ion flow. In human, real-time RT-PCR reveals that claudin-2 and -15 are predominantly expressed in the proximal parts of the GI tract.⁵ By immunofluorescence staining, claudin-1 is expressed and mainly localized at the apex of epithelial cells with a typical reticular pattern in the colon.^{6,7} Claudin-2 is detected in both villus and crypt cells of the small intestine but restricted to undifferentiated crypt cells in the colon. Claudin-3, -4, -7 and -8 are predominantly expressed in the distal parts (colon, sigmoid and rectum) of the GI tract. Claudin-12 shows an ubiquitous expression pattern throughout the entire GI tract.⁵ Claudin-4 and -7 are detected in both the lateral membrane of the cell surface and the TJs.⁸

In mice, mRNAs of claudins 1–5, 7–15, 17 and 18 are all detected in GI tract with claudin-2, -3, -7 and -15 being the most highly expressed while claudin-1, -5, -9, -10 and -11 being

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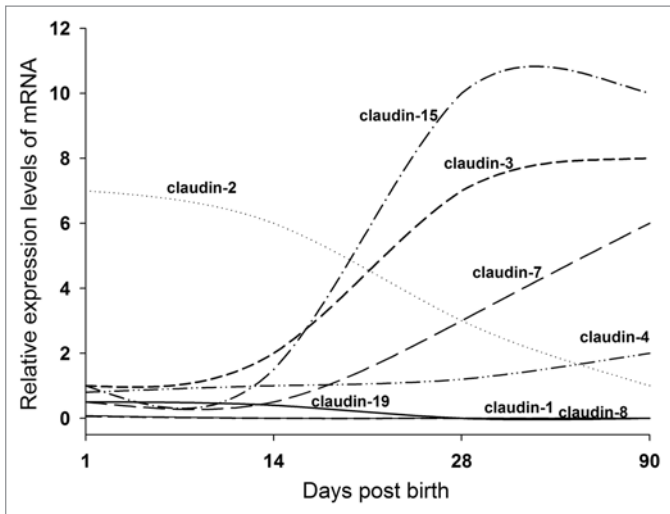
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Table 1. Expression of claudins in mammalian intestines

Intestine segments	Expression of claudins in human	References	Expression of claudins in mice	References
duodenum	1, 2, 3, 4, 5, 7, 12, 15,	5, 79, 80	2, 3, 7, 15	9, 32
jejunum	Not determined	5	2, 3, 7, 12, 15	9, 12, 13, 32
ileum	2, 7, 8, 12, 15	5	1, 2, 3, 4, 7, 8, 12, 15	9, 10, 31, 103, 167
Large intestine	1, 3, 4, 7, 8, 12, 15	5, 6, 7, 8	1, 2, 3, 4, 5, 7, 8, 12, 13, 15	9, 32, 41, 47, 49, 77, 96

**Figure 1.** Claudin profiling during postnatal intestinal development in mice (adapted from Holmes et al., 2006).⁹

weakly expressed.^{9,10} Both claudin-7 mRNA and protein are strongly expressed in the duodenum, jejunum, ileum and colon.¹¹ Claudin-8 mRNA and protein are moderately expressed in the ileum and colon, but were absent in the jejunum and duodenum. Claudin-12 mRNA and protein are highly expressed in the ileum and are moderately observed in the jejunum and colon and barely detected in the duodenum. Claudin-13 mRNA and protein are observed in colon, but undetectable in the rest of the intestines. The expression of claudin-15 mRNA and protein is high in the duodenum and jejunum, but is decreased from the ileum to colon.¹² Claudin expression profiles in both human and mice are shown in **Table 1**.

In terms of sub-cellular localization, claudin-2 protein is localized in the deep crypt of the distal colon while claudin-10 is localized in the entire crypt.⁹ Claudin-7 is expressed along apical and basolateral cell surfaces of epithelial cells in small intestines, while claudin-8 is distributed on basolateral membranes of epithelial cells in the ileum and colon.^{11,12} Claudin-12 is localized at the apical-most tips of lateral membranes of epithelial cells in the jejunum, ileum and colon, but not in the duodenum. Claudin-13 is distributed at the apical membranes of colonic epithelial cells and claudin-15 is localized at the apical membranes of epithelial cells in both the small and large intestines.^{12,13}

Peyer's patches are important sites for mucosal immune responses in intestines, and it contains follicle-associated epithelium (FAE) structure. Claudin-1 is detected in the TJs of intestinal lymphoid FAE in mice;¹⁴ claudin-2 is only weakly expressed

on the crypt side of the FAE compared with stronger expression on the crypt side of villous epithelial cells; claudin-3 is found throughout the dome of the FAE; claudin-4 is preferentially expressed in the apex region of the FAE in mice.¹⁵

The expression of claudins also changes during the postnatal development. During the development, rodent intestinal barrier function matures in the first 3 weeks of life due to the establishment of the intestinal flora. Commensal bacterial colonization induces intestinal barrier function maturation by promoting claudin-3 expression in mice.¹⁶ In mice, by RT-PCR, claudin-19 is only detected at days 1, 14 and 28. Claudin-2 expression decreases continuously from birth to 90 d by 10-fold, while claudin-3, -4, -7 and -15 increase in the range of 2- to 20-fold. Claudin-1 and -8 fall dramatically after day 1 and claudin-12 doubles between day 14 and 28 (**Fig. 1**).⁹

The rat colon shows the highest epithelial resistance, followed by duodenum, jejunum and ileum. Duodenum and colon have the strongest expression of "tightening" claudin-1, -3, -4, -5 and -8 and the lowest expression of claudins mediating ion permeability, mainly claudin-2, -7 and -12, compared with jejunum and ileum.¹⁷ Claudin-3, -4 and -5 are all detectable in ileum, but only claudin-3 and -5 are detected in jejunum in rats. Claudin-4 expression is also found in the enteric neurons in rat distal colon.¹⁸ The localization of claudins can be junctional, lateral or show a gradient in junctional and/or lateral distribution along the crypt-to-villus surface axis. Claudin-2 is restricted to crypts in the small and large intestines. Claudin-3 localizes both at the TJs and the lateral membrane and increases from the crypt to surface cells of the colon. Claudin-4 expression is highest in the lateral membrane of the villus surface epithelial cells in both the small and large intestines. Claudin-5 is strictly located at the junctions of both endothelial and epithelial cells and shows no gradation along the crypt-to-villus surface axis in any part of the intestines. Claudin-2 shows a crypt-to-villus decrease.¹⁹

In dogs, there are high expressions of claudin-3 and -5 and a weak expression of claudin-7 in the duodenum. In the colon, there are high expressions of claudin-2 and -3 and weak expressions of claudin-5 and -7 proteins by western blot. The localization detected by immunofluorescence microscopy shows that the duodenum and colon have the staining for claudin-3 and -5 in the most apical region and claudin-7 in the basolateral region.²⁰

Studies of claudin mutations in humans and gene-knockout in mice have revealed specific roles for a number of claudins in the TJ barrier function, selective ion permeability, as well as their related pathological phenotypes.

Claudin-7 forms a protein complex with claudin-1 and integrin $\alpha 2$ at the basolateral surface in mouse intestines. Knockout of claudin-7 in mice (Cldn7^{-/-}) has severe intestinal defects

resembling inflammatory bowel disease (IBD) syndrome that includes mucosal ulcerations, epithelial cell sloughing and inflammation, which lead to the death of the mice. At the molecular level, *Cldn7^{-/-}* intestines produce significantly higher levels of cytokines, NF- κ B p65 and cyclooxygenase 2 (COX-2) as well as matrix metalloproteinase (MMP)-3 and -7.¹¹ Claudin-8 is found to be involved in regulating paracellular Na⁺ permeability, protecting the leakage of Na⁺ into the intestinal lumen.²¹ Claudin-2 and -12 contribute to Ca²⁺ absorption in intestinal epithelial cells. The expression of claudin-2 and -12 could be induced by an active form of vitamin D₃, 1 α , 25-dihydroxyvitamin D₃.^{22,23} The small intestine is responsible for nutrient absorption and the absorption of monosaccharides, amino acids and vitamin C is coupled directly to Na⁺ absorption. It has been reported that claudin-15 knockout mice are born and grow normally but with an enlarged upper small intestinal phenotype, megaintestine, in that the upper small intestine is approximately 2 times larger than normal in length and diameter. Knockout of claudin-15 does not alter the expression of other types of claudins, such as claudin-1, -2, -3, -4, -7, -12, -18, -20 and -23.²⁴ Double knockout of claudin-2 and -15 reduces the paracellular flow of Na⁺ from intestinal submucosa into the lumen and decreases the absorption of glucose, amino acids and fats, thus leads to the malnutrition and death of the mice 25 d after birth.²⁵ Claudin-16 may be responsible for the defective absorption of Ca²⁺ in the intestines causing primary hypercalciuria.²⁶

The function of claudins in intestines just begins to be unraveled in recently years. It is expected that new discovery will be made when more claudin-specific gene deletion models become available in the near future.

Regulation of Claudin Expressions in Intestines

The expression levels of claudins in intestines are under the regulation of a number of genes and/or proteins. Cathepsin L belongs to the cysteine protease class of the papain superfamily that is involved in intracellular and extracellular protein degradations. Inhibition of intracellular cathepsin L activity results in a rapid upregulation of claudin-1 protein accumulation in IEC-6/*Cdx2L1* cell line, an epithelial cell line conditionally expressing *Cdx2*, a transcription factor that initiates intestinal epithelial differentiation. Mutant mice defective in cathepsin L activity display an elevated level of intestinal claudin-1 and -2 expression, which may be responsible for the intestinal neoplasia in mice.²⁷ Claudin-1 gene is also under the regulation of β -catenin. Claudin-1 expression decreases significantly in response to the reduction of β -catenin in intestines.²⁸ The trefoil factor family 3 (TFF3) plays an important role in the protection and repair of the GI mucosa. Overexpression of TFF3 in HT29/B6 cells, a human colon carcinoma cell line, increases the cellular level of claudin-1 and decreases the amount of claudin-2 accompanied by an increase in the TER in confluent monolayers of these cells.²⁹ Claudin-2 expression is under the positive regulation of *Cdx2*.³⁰ The 5'-flanking region of the claudin-2 gene contains binding sites for intestine-specific Cdx homeodomain proteins and hepatocyte nuclear factor (HNF)-1, which are conserved

in human and mouse. Both *Cdx1* and *Cdx2* activate the claudin-2 promoter in the human intestinal colorectal adenocarcinoma epithelial cell line *Caco-2*, which is a widely used model for studying the human intestinal barrier. HNF-1 α augments the *Cdx2*-induced but not *Cdx1*-induced transcriptional activation of the human claudin-2 promoter.³⁰ Claudin-2 expression in the intestine is also regulated by the transcriptional factor GATA-4, which is undetectable in the colon.³¹ Claudin-2, -3, -7 and -15 have been found to be recruited to the TJs in the mouse intestinal epithelial cells by EpCAM. Mutation of EpCAM in mice leads to the downregulation of these claudins and the disruption of TJ strands.³²

Sodium proton exchangers (NHEs) constitute a large family of integral membrane protein transporters that are responsible for the counter-transport of protons and sodium ions across lipid bilayers. Mucosa from Na⁺/H⁺ exchanger 2 knockout mice subjected to complete mesenteric ischemia displays a shift of occludin and claudin-1 membrane expression to cytoplasm and shows the disruption of occludin and claudin-1 localization patterns following injury.³³ Expression of claudin-2 may be under the regulation of NHE3. Knockout of NHE3 in mice decreases the mRNA expression of claudin-2 and -15.³⁴ Aquaporin 3 (AQP3) knockdown significantly enhances the paracellular permeability and decreases the expression of claudin-1 and occludin by western blots.³⁵

Protein tyrosine phosphatase, non-receptor type 2 (PTPN2) is an inflammatory bowel disease (IBD) candidate gene. Mutation of PTPN2 causes IBD. McCole has reported that PTPN2 protects the epithelial barrier function by restricting the capacity of IFN- γ to increase epithelial permeability and prevent the induction of the pore-forming protein, claudin-2 expression.³⁶ Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system as well as in the digestive system. TLR2^{-/-} mice suffer the impaired epithelial barrier function with colonic mucosal ulcerations, bleeding and increased cell death after bacterial pathogen *Citrobacter rodentium* infection compared with the wild-type (WT) mice. Claudin-3 is mislocalized in TLR2^{-/-} intestine after infection in that it relocates from the normally lateral distribution to the cytoplasm in the enterocytes.³⁷ IL-10 knockout mice (IL-10^{-/-}) exhibit a significant increase in the cumulative permeation of mannitol through the colonic mucosa and a significant decrease in TER compared with WT mice. Histological analysis shows that spontaneous colitis develops in all IL-10^{-/-} mice with mucosal ulceration, erosion and neutrophil infiltration in the lamina propria. Molecular analysis reveals the decreased expression and redistribution of claudin-1, ZO-1 and occludin in IL-10^{-/-} mice. Probiotic *Lactobacillus plantarum* treatment on IL-10^{-/-} mice greatly improves colitis in that the paracellular permeability is reduced and the expression and distribution of claudin-1, ZO-1 and occludin is restored in IL-10^{-/-} mice.³⁸

FoxO4 is a member of the forkhead box transcription factor O (FoxO) subfamily. FoxO4 inhibits the transcriptional activity of NF- κ B and protects the mice against colonic injury and inflammation. FoxO4 deficiency in mice results in an increase in intestinal epithelial permeability and the downregulation of ZO-1 and claudin-1.³⁹ Guanylyl Cyclase C (GCC) signaling

Table 2. Regulation of claudin expression in intestines by transcription factors or other protein modulators

Claudin	Transcription factors	Other protein modulators	References
1	β -catenin, Trefoil factor family 3, FoxO4, Smad5, COX-2	Cathepsin L, Na ⁺ /H ⁺ exchanger 2, Aquaporin 3, IL-10, Muc2	27, 28, 29, 33, 35, 38, 39, 43, 48, 49
2	Trefoil factor family 3, Cdx1, Cdx2, HNF-1 α , GATA-4, Smad5	Cathepsin L, Na ⁺ /H ⁺ exchanger 3, EpCAM, PTPN2, Guanylyl cyclase C, Matriptase, ST14	27, 29, 30, 31, 32, 34, 36, 40, 43, 51, 53
3	---	EpCAM, TLR2	32, 37
4	TGF- β	Guanylyl cyclase C, PrP(c), Connexin 26	41, 46, 52, 175
5	---	Muc2	49
7	---	EpCAM	32
10	---	Muc2, JAM-A	49, 54
15	---	EpCAM, Na ⁺ /H ⁺ exchanger 3, JAM-A	32, 34, 54

is a critical mediator of intestinal fluid homeostasis. Knockout of GCC in mice results in increased intestinal permeability. Claudin-2 expression is found to be reduced in GCC deficient intestine.⁴⁰ Lin et al. have reported that mice deficient in GCC exhibits the intestinal barrier hyper-permeability associated with the reduced junctional proteins occludin and claudin-4. The same observation is also found in Caco-2 cells. This regulation is possibly through the upregulation of AKT1 pathway. Restoration of occludin and claudin-4 is associated with the reconstitution of intestinal barrier integrity by reducing AKT1 expression in GCC knockout mice.⁴¹ Claudin-1 and -4 can be phosphorylated by PKC θ and they can also form endogenous complexes with PKC θ , which has the stabilizing effect on the monolayer barrier dynamics in Caco-2 cells.⁴²

Loss of smad5 leads to the de-regulation of claudin-1 and -2 expression in intestines.⁴³ Knockdown of PTEN significantly inhibits the polarization, functional differentiation and brush border development in Caco-2/15 cells, a stable clone of the parent Caco-2 cells. A strong reduction in claudin-1, -3, -4 and -8 is also observed in addition to a decrease in TER.^{44,45} Claudin-4 has been found to be associated with cellular prion protein (PrP(c)) that is located at cell-cell junctions. Knockout of PrP(c) in mice greatly increases the paracellular permeability. Knockdown of PrP(c) in Caco-2/TC7 enterocytes, a stable clone of the parent Caco-2 cells, decreases the expression of claudin-4, as well as other TJ proteins, such as occludin, ZO-1 and tricellulin at cell contacts.⁴⁶ The protein C (PC) pathway is a well-characterized coagulation system. PC deficient mice develop spontaneous intestinal inflammation and show increased intestinal permeability. Structural analysis of epithelial TJ molecules reveals that lack of PC leads to decreased JAM-A and claudin-3 expression and altered pattern of ZO-1 expression.⁴⁷ Knockout of COX-2 significantly increases epithelial permeability and reduces the expression of claudin-1, ZO-1 and occludin in the ileum following cecal ligation and puncture in mice.⁴⁸ The TJ-associated gene claudin-10 is upregulated, whereas claudin-1 and -5 are downregulated in Mucin Muc2 knockout mice compared with the WT.⁴⁹

Claudin expression levels are also under the regulation of other proteins, such as desmoglein 2,⁵⁰ matriptase,⁵¹ connexin 26,⁵² suppression of tumorigenicity-14 (ST14),⁵³ and JAM-A.⁵⁴ Proteins and their regulatory effect on the expression of claudins in intestines are summarized in **Table 2**. The search for the

effective regulators of claudin genes/proteins is an ongoing task and has high clinical relevance.

Claudins are Modulated in the Disease State of Intestines

The intestinal barrier plays a critical role in the transport of nutrients and macromolecules. At the same time, it has to provide an effective barrier to harmful macromolecules and microorganisms.⁵⁵ Defects in this barrier function have been observed in intestinal disorders, such as IBD that includes ulcerative colitis (UC) and Crohn disease (CD), food allergies and celiac diseases. TJs are essential components of the physical intercellular barrier that separates and prevents the mixing of luminal contents with the abdomen. Compromised epithelial barrier function and TJ alterations are hallmarks of a number of GI disorders. Luminal antigen uptake occurs via TJ discontinuities and epithelial gross lesions, which is likely to induce many other changes to the epithelium besides simply TJ barrier alterations.

In IBD, epithelial barrier function is impaired, which contributes to diarrhea by a leak flux mechanism and triggers inflammation by an increased luminal antigen uptake. Studies show that claudin-1 protein expression is significantly reduced in IBD patients and its expression is correlated with the duration of the IBD symptoms.⁵⁶ Further studies reveal that the expression of claudin-1 is downregulated in epithelial cells immediately adjacent to transmigrating neutrophils in IBD.⁵⁷ The localization of claudin-1 is also irregularly distributed and disappeared from intercellular junctions in the samples from irritable bowel syndrome (IBS) patients.⁶ There are conflicts on the expression levels of claudin-3 and -4 in IBD patients. Claudin-3 and -4 are present throughout normal colonic epithelium, but are unchanged, reduced or redistributed in the disease surface epithelium.⁵⁸ Some studies show reduced expression of claudin-3 with no changes in claudin-4 expression,⁵⁹ while others report stable claudin-3 expression with reduced claudin-4 expression at protein levels,^{8,60} or without any changes,^{58,61} or changes only in the cellular localization.⁵⁸ IBD-associated diarrhea may result from NF- κ B-mediated TJ proteins occludin, claudin-1 and ZO-1 internalization, thus increasing the paracellular permeability.⁶² Disruption of the epithelial barrier is also associated with the internalization of claudin-4 to a sub-apical cytoplasmic compartment.⁵⁶

Epithelial barrier function is impaired in UC. Claudin-2 is undetectable in normal colon, but it is strongly expressed along the inflamed crypt epithelium. A $956 \pm 252\%$ increase in claudin-2 expression is observed in mucosal biopsy specimens from human patients with UC.⁶³ Mennigen et al. have demonstrated that expressions of claudin-1, -3, -4 and -5 are all decreased in acute colitis.⁶⁴ Urinary claudin-3 can be used as an early noninvasive diagnostic marker for intestinal TJ loss. It is correlated with reduced claudin-3 staining in colonic tissues from biopsies.⁶⁵ T helper (Th) 2 cytokines, such as interleukin (IL)-13 and tumor necrosis factor (TNF)- α , are important factors in UC. IL-6, a pleiotropic cytokine, is elevated in IBD patients. IL-6 treatment increases TJ permeability by stimulating the expression of channel-forming claudin-2 in intestinal epithelial Caco-2 cells.⁶⁶ IL-13 stimulates epithelial apoptosis as well as upregulates claudin-2 in UC.^{67,68} This activation is through the signal transducer and activator of transcription 6 (STAT6) pathway.⁶⁹ Myosin light-chain kinase (MLCK) activation that is often observed in IBD patients can also trigger claudin-2 synthesis and increase paracellular cation flux.⁷⁰ Claudin-7 is detected in both the lateral membrane of the cell surface and the TJs by immunofluorescence staining and its signal is decreased in active UC biopsies collected from the rectum of patients. Downregulation of claudin-7 may lead to an altered TJ structure and thus the impaired epithelial function in active UC.⁸ Chronic inflammation in mucosal tissues can influence the epithelial barrier function via pro-inflammatory cytokines such as interferon (IFN)- γ and TNF- α . IFN- γ induces a time-dependent increase in paracellular permeability that is associated with the internalization of claudin-1, occludin and JAM-A.⁷¹

The epithelium in inflamed intestinal segments of patients with CD is characterized by a reduction of TJ strands, strand breaks and alterations of TJ protein content and composition.⁷² CD patients show significantly increased intestinal permeability. In the intestines of patients with active CD, freeze fracture electron microscopic analysis reveals that occludin and the sealing TJ proteins claudin-5 and -8 are all downregulated and redistributed off the TJ, whereas the pore-forming TJ protein claudin-2 is strongly upregulated, which constitute the molecular basis of TJ changes.⁵⁹ Das et al. have also reported that claudin-4, -5 and -8 are redistributed from the TJ to the subjunctional lateral membrane in CD patients.⁷³

The mRNA of claudin-12 is significantly upregulated in the ileum of CD patients while the claudin-2 mRNA is significantly reduced by 5-fold in the sigmoid colon compared with healthy controls. The mRNA expression levels of claudin-12 and -4 are significantly downregulated in CD patients in the colon compared with the nearby unaffected tissue. Although significant differences are not observed, there is a trend of downregulation of claudin-3 in the colon of CD patients.⁵ Portitz et al. have reported an increase of claudin-1 in human UC specimens and unchanged claudin-1 expression in CD patients. Thus it is suggested that claudin-1 may be used to differentiate between UC and CD cases.⁷⁴

Various animal models are established to study the mechanisms causing IBD. An upregulation of claudin-2 is observed in

dinitrobenzenesulfonic acid (DNBS)-induced colitis in mice.⁷⁵ The 2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced colitic rats show damaged intestinal mucosa and decreased expression of claudin-1, -3, -5 and -8 in TJ membrane fractions. The n-3 polyunsaturated fatty acids (PUFAs) can prevent the redistribution of these TJ proteins and elevate the expression of claudin-1, -5 and -8.⁷⁶ DSS-treated mice/rats show colitis-like syndrome with inflammatory response and mucosal damage and are often used as a model to study IBD. Immunohistochemistry staining as well as western blots show the decreased signals of colonic claudin-1, -3, -5, -7 and -8 after DSS treatment in mice, while claudin-2 expression is significantly increased after DSS treatment. These changes are restored and the intestinal damage is attenuated after AMD3100 treatment. AMD3100 is a CXCR4 antagonist. CXCR4 is constitutively expressed in intestinal epithelial cells and its expression is increased in the specimens of UC patients.⁷⁷ SAMP1/YitFc (SAMP) mouse strain is a spontaneous model of IBD, closely resembling CD. SAMP mice show the disrupted barrier function accompanied by the aberrant expression of claudin-2 and occludin.⁷⁸

Gluten-intolerant diseases include celiac disease and gluten-sensitive disease. Celiac disease is an autoimmune enteropathy triggered by the ingestion of gluten. Gluten-sensitive individuals develop GI symptoms similar to those in celiac disease, but the overall clinical symptom is generally less severe. Gluten-sensitivity displays significantly reduced intestinal permeability and significantly increased expression of claudin-4 at the mRNA level compared with the healthy controls in adults.⁷⁹ Biopsies from proximal and distal parts of duodenum from children with celiac disease show that claudin-2 and -3 expression are significantly increased in the severe form of celiac disease in bulb and in distal duodenum in comparison to controls, which may be, at least in part, responsible for increased permeability observed in celiac disease patients.⁸⁰ In celiac disease patients, pore-forming claudin-2 and -15 are upregulated and the tightening claudin-3, -5 and -7 and occludin are downregulated by western blot.⁸¹ Patients with refractory celiac disease unresponsive to the treatments show increased expression of claudin-4 and -5 and decreased expression of pore-forming claudin-2.⁸² Recent studies have indicated that schizophrenia may be also related to the celiac disease in that the celiac disease causes an alteration of gut permeability that may allow the exogenous psychosis-causing substances to enter the body thus causing the development of schizophrenia and other mental conditions.⁸³ Collagenous colitis is an inflammatory disease of unknown etiology with diarrhea as the leading symptom. A pronounced decrease in NaCl absorption is observed in collagenous colitis, which may be due to the downregulation of occludin and claudin-4.⁶⁰

Besides IBD, there are a number of diseases that have been found to be related to intestinal dysfunction. The *in vitro* culture model of distal duodenum biopsies from nine patients with food allergy is established to study the food allergy. Peptic-tryptic digest of wheat gliadin, wheat albumins and apple proteins are applied on the culture of biopsy tissues to induce food allergy. For the allergic tissues, after exposure to food allergens, claudin-1 expression appears to be reduced and exhibits breaks at the

Table 3. Changes in claudin expression in intestines in diseases

Diseases	Changes of claudin(s)	References
inflammatory bowel disease	1 ↓	56, 57
ulcerative colitis	2 ↑, 7 ↓	8, 63
acute colitis	1 ↓, 3 ↓, 4 ↓, 5 ↓	64
Crohn's disease	2 ↑, 4 ↓, 5 ↓, 8 ↓, 12 ↑	5, 59
gluten-sensitive disease	4 ↑	79
celiac disease	2 ↑, 3 ↓, 5 ↓, 7 ↓, 15 ↑	80, 81
collagenous colitis	4 ↓	60
food allergy	1 ↓	84
acute pancreatitis	1 ↓, 4 ↓	85
chronic kidney disease	1 ↓	86
liver cirrhosis	1 ↓	87
obstructive jaundice	1 ↓, 4 ↑, 7 ↓	91
strangulated intestinal obstruction	1 ↑	7
ischemia/reperfusion injury	1 ↑, 4 ↑, 7 ↓	101
burn injury	1 ↑	103

cell-cell regions.⁸⁴ Acute pancreatitis can induce intestinal injury and reduce the expression of claudin-1 at both mRNA and protein levels in the intestinal tissues. Studies of colonic biopsies indicate that both minimized and conventional cardiopulmonary bypass procedures cause moderate mucosal damage, an increase in epithelial cell proliferation and a decrease in expression of TJ protein claudin-4.⁸⁵ Other diseases, such as uremia and chronic kidney disease (CKD) can affect intestinal barrier function and cause systemic inflammation. CKD greatly reduces the protein expressions of claudin-1, occludin and ZO-1 in the colonic mucosa, showing the thickened colonic wall and heavy infiltration of mononuclear leukocytes in the intestinal lamina propria.⁸⁶ Patients with liver cirrhosis show the intestinal barrier dysfunction and hyperpermeability. Assimakopoulos et al. have recently reported that liver cirrhosis patients show significantly reduced expression of occludin and claudin-1 in duodenum as compared with healthy controls by immunohistochemical analysis. As a result, liver cirrhosis patients present significantly higher endotoxin values in peripheral blood as compared with healthy controls. Disruption of TJs could be due to the cytokine secretion, such as TNF- α and IFN- γ , in cirrhosis patients.⁸⁷ Iron can be toxic to the intestines in that it increases cell monolayer permeability and causes partial delocalization of claudin-4 from the plasma membrane to an intracellular compartment.⁸⁸ Expression of claudin-2 is induced by iron-deficiency in rats' duodenum.⁸⁹ High fat diet is also shown to increase small intestinal permeability that is possibly due to decreases in claudin-1, claudin-3 and occludin expression in the small intestine.⁹⁰ Intestinal hyper-permeability is often seen in patients with obstructive jaundice. Occludin, claudin-1 and -7 are significantly decreased whereas claudin-4 is significantly increased in jaundiced patients and their distributions are altered as well. Patients with malignant obstructive jaundice show loose microvillus and wider cell junctions with reduced expression of claudin-1 and increased

expression of claudin-4 in the duodenum.⁹¹ Strangulated intestinal obstruction (STR-IO) patients show increased expression levels of ZO-1, occludin and claudin-1 at both mRNA and protein levels in the colonic tissues.⁷ Chronic metabolic acidosis (CMA) enhances the mRNA expressions of claudin-2, -3, -6, -8, -11, -12, -14, -19 and -22 in Sprague-Dawley rats with chronic metabolic acidosis induced by NH₄Cl.^{92,93} Hemorrhagic shock (HS) leads to the intestinal barrier loss as well as the loss of claudin-3 and ZO-1 in rats.⁹⁴ Beutheu et al. have shown that claudin-1 and occludin expression levels are reduced during the acute phase of mucositis compared with control rats. During the recovery phase, their expression levels are restored.⁹⁵ Wang et al. have reported that the side-stream smoking increases mouse intestinal bacteria and upregulates the expression of claudin-3 and ZO-2 in mouse large intestine.⁹⁶

The intestinal epithelial barrier function is often disrupted in many surgical diseases, including trauma, shock, burn injury and the other surgically critical illness, resulting in the increased intestinal permeability and subsequent translocation of bacteria and/or endotoxin from the GI tract.^{97,98} Intestinal surgical trauma can transiently compromise the protein levels of claudin-1 and E-cadherin in intestines.⁹⁹ Ischemia/reperfusion (I/R) injury of the intestine is the leading cause of organ dysfunction after restoration of blood flow in many diverse events, including shock and intestinal transplantation. I/R injury can cause intestinal mucosal lesions in rats and redistribute occludin, ZO-1, claudin-1 and -3 from the cell membrane to the cytoplasm in intestinal epithelial cells as shown by immunofluorescent microscopy.¹⁰⁰ In addition, mRNA expression levels of claudin-1 and -7 are significantly decreased from the beginning of reperfusion and are recovered to the control level by 24 h. On the other hand, claudin-4 mRNA expression level is significantly increased from the start of the reperfusion and then reaches to the control level 24 h after reperfusion. The protein expression levels of claudin-1, -2, -4 and -7 are significantly decreased until 1 h after reperfusion.¹⁰¹ The expression level of nitric oxide (NO) is increased during intestinal I/R. NO participates in opening TJ by down-regulating the protein expression of claudin-1, -2, -4 and -7.¹⁰²

Claudin-1 is predominantly localized in the cytoplasm of the ileum in mice, and its expression is markedly elevated in cytoplasm following burn injury, which may contribute to the histological damage of intestinal mucosa after burn injury.¹⁰³ EtOH combined with burn injury results in no change of claudin-1 protein content but its phosphorylation on tyrosine is decreased following EtOH and burn injury.¹⁰⁴ **Table 3** summarizes the changes of claudin expression in intestines in currently known disease status. We conclude that the majority of the claudins are downregulated in the disease with only pore-forming claudin-2 being consistently upregulated in several diseases.

Claudins and Colorectal Cancer

The function of TJ proteins is compromised not only in a number of intestinal diseases but also in colorectal cancer. Claudin-1 is weakly expressed at the apical boarder of the lateral membrane of normal enterocytes, but is strongly expressed at cell-cell

boundaries as well as in the cytoplasm of colorectal cancer cells.²⁸ Huo et al. have also indicated that the expression of claudin-1 at the mRNA and protein levels is found to be increased in colorectal cancer tissue in comparison to that in the normal tissue specimens. The mRNA level of claudin-1 is correlated with tumor depth. Claudin-1 protein may therefore be one of the major factors involved in colorectal tumorigenesis.¹⁰⁵ The increased expression of claudin-2 may participate in colorectal carcinogenesis.¹⁰⁶ Claudin-3 is overexpressed in human cancerous colorectal tissues and may be used as a biomarker to differentiate cancerous colorectal tissues from normal colorectal tissues.¹⁰⁷ Using tissue biopsy samples, Mees et al. have also found that colorectal carcinoma in human exhibits significantly elevated expression levels of claudin-1, -3, -4 compared with normal mucosa, which suggests that these proteins may be potential markers for diagnosing colorectal carcinoma.¹⁰⁸ Claudin-7 overexpression is found in colorectal cancer. Its overexpression is under the regulation of Tcf-4 and Sox-9.¹⁰⁹ Claudin-8 is downregulated in colorectal adenoma samples compared with the normal intestinal tissues.¹¹⁰ Plasma markers for enterocyte damage (I-FABP, I-BABP) and urinary presence of TJ protein claudin-3 can be used to assess the gut mucosal barrier.¹¹¹

Effects of Bacteria/Viruses on Claudin-Mediated Intestinal Functions

A number of bacteria and viruses can cause the infection of intestines and alter the intestinal permeability. While some of the bacteria have beneficial effects to the intestines, it has been recognized that bacteria and/or endotoxin translocation and increased gut permeability play a very important role in the setting of severe complications such as systemic inflammatory response syndrome, sepsis, multiple organ dysfunction syndrome and multiple organ failure.^{112,113}

It is known that *Enteropathogenic Escherichia coli* (EPEC) infection disrupts TJs in mice. Occludin and claudin-1 are displaced from TJ membrane microdomains to cytoplasm after EPEC infection using sucrose density gradient centrifugation and western blot analysis.¹¹⁴ It has been reported that the micro integral membrane protein (MIMP) can protect the intestinal barrier from injury by EPEC infection since MIMP-expressing NCM460 epithelial cells (NCM460/MIMP) show significantly higher immunostaining signals of occludin, claudin-1, JAM-1 and ZO-1 compared with parental NCM460 cells.¹¹⁵ The associations between ZO-1, occludin and claudin-1 are progressively decreased after EPEC infection in human intestinal epithelial T84 cells, resulting in a loss of barrier function.¹¹⁶

Enterotoxigenic Escherichia coli (EAEC)-infected T84 cells exhibit irregular shapes and some cells become elongated and/or enlarged. Infection of EAEC on intestinal T84 cells also induces a decrease in TER and dissociation of claudin-1 from the TJs.¹¹⁷

Enterohemorrhagic Escherichia coli (EHEC) infection leads to microvillous effacement of mouse colonocytes. EHEC infection results in the alteration of TJ proteins claudin-2 and -3. After infection, claudin-3 immunostaining is clearly diminished from the lateral membranes. However, claudin-2 staining

is progressively increased within the cytoplasm. Quantitative real-time PCR shows that EHEC alters the mRNA transcription of claudin-2 and -3. Most notably, claudin-2 expression is significantly increased, which correlates with increased intestinal permeability.¹¹⁸

Yersinia enterocolitica is a common cause of acute gastroenteritis. Exposure of human colonic HT-29/B6 cells to *Y. enterocolitica* results in a decrease in TER. After infection, claudin-3, -4 and -8 are redistributed from TJ into the cytoplasm. In addition, the expression of claudin-2, -3, -8, -10 and ZO-1 is diminished by western blot analysis.¹¹⁹

Campylobacter jejuni is a leading cause of human enterocolitis. Infection of polarized T84 monolayers with *C. jejuni* causes a time-dependent decrease in TER. Lipid rafts are submembrane domains that are preferentially partitioned in the apical membrane of polarized epithelium. The loss of TJ barrier function has been correlated with translocation of lipid raft-associated TJ proteins.¹²⁰ In the uninfected control T84 cells, the majority of claudin-1 is found in lipid rafts. Upon infection, T84 cells exhibit a concomitant increase in raft-associated claudin-1.¹²¹ Infection of HT-29/B6 cells with bacteria strains *Campylobacter concisus* impairs the epithelial barrier function characterized by a time- and dose-dependent decrease in TER. The expression level of barrier-forming TJ protein claudin-5 is only 66 ± 8% at the protein level and 49 ± 16% at the mRNA level compared with the control cells after infection. However, there is no distribution change of claudin-5 observed.¹²²

Humen et al. have reported recently that *Giardia intestinalis trophozoite* promotes an adhesion-dependent decrease in TER accompanied by a rearrangement of functional TJ-associated occludin and delocalization of claudin-1 in human intestinal Caco-2/TC7 cells.¹²³ *Entamoeba histolytica* produces and secretes Prostaglandin E(2) (PGE(2)), an inflammatory molecule that decreases barrier integrity of TJs. Loss of mucosal barrier integrity corresponds with the increased Na⁺ permeability across TJs. PGE(2) also dissociates claudin-4 from cell membrane to cytoplasm.¹²⁴

Bifidobacterium bifidum improves the intestinal integrity in a rat model of necrotizing enterocolitis. Asphyxia and cold stress are applied to the premature newborn rats to induce neonatal necrotizing enterocolitis (NEC). Claudin-3 is significantly increased in NEC rats and is localized mainly in the cytoplasm of the enterocytes. Administration of probiotic *B. bifidum* normalizes the expression and localization of claudin-3 in the ileum compared with the control animals with NEC, thus it protects against NEC in the neonatal rat model.¹²⁵ *Bifidobacterium infantis* culture media increase TER and the expression of occludin and ZO-1 while decreasing claudin-2 in T84 cells.¹²⁶

Lactobacillus plantarum inhibits the intestinal epithelial barrier dysfunction induced by unconjugated bilirubin (UCB). High concentrations of UCB cause cytotoxicity and decrease the TER of the Caco-2 cell monolayer. UCB treatment results in a loss of claudin-1 and -4 from the cell membrane as revealed by immunofluorescence microscopy. Both protein and mRNA levels of claudin-1 and -4 are reduced in UCB-treated cells as compared with the control cells. Probiotic *L. plantarum* exerts a protective effect

against UCB damage to Caco-2 monolayer cells, and it restores the structure and distribution of TJ proteins and increases the mRNA and protein levels of claudin-1 and -4.¹²⁷ *L. plantarum* also prevents the reduced expression and rearrangement of claudin-1, occludin, JAM-1 and ZO-1 proteins induced by enteroinvasive *Escherichia coli* (EIEC) infection in Caco-2 cells.¹²⁸

Salmonella typhimurium regulates the distribution of claudin-1 from the Triton X-100-insoluble fractions to the Triton X-100-soluble fractions. Infection with *S. typhimurium* is associated with the rapid targeting of TJ complex and loss of barrier function. These events result in the enhanced bacterial translocation and initiation of polymorphonuclear leukocyte (PMN) migration across the intestinal barrier.¹²⁹ Analysis of duodenal biopsy specimens from patients with chronic giardiasis *Giardia lamblia* infection shows the downregulation of claudin-1 and increased epithelial apoptosis.¹³⁰ *Shigella flexneri* serotype 2a has the ability to remove claudin-1 from TJs upon exposure to T84 cell monolayers.¹³¹

The neonatal small intestine is susceptible to the damage by endotoxins and LPS is one of the bacteria endotoxins that are widely used by researchers to establish the intestine damage model. Bacteria endotoxins are the main cause of sepsis, which is one of the primary causes of patient death in the intensive care units. Significantly damaged intestinal tissues, inflammatory cell infiltration and hemorrhage are observed in the sepsis patients. During the polymicrobial sepsis, TJ architecture and the protein redistribution in TJ membrane microdomains are altered.¹³² ZO-1 and claudin-2 expressions are lost from the cell membrane, and their protein expression levels are decreased after LPS treatment in rats. Carbachol, one of the clinical cholinomimetic drugs, decreases the mucosal damage and ameliorates the intestinal epithelial TJ damage induced by LPS in that the gut barrier permeability is reduced, and the ultrastructure disruption of TJs is prevented. Carbachol treatment also significantly increases ZO-1 and claudin-2 protein expression after LPS administration.¹³³ Claudin-1, -3, -4, -5 and -8 are present predominantly in the microvillous surface and along the lateral membranes of the epithelial cells. Polymicrobial sepsis leads to the diffusion and loss of these TJ proteins from the lateral cell boundaries in mice. However, the expression of claudin-2 is markedly upregulated in sepsis by immunofluorescence staining.

Rotavirus infection of Caco-2 cells alters paracellular permeability and causes redistribution of TJ proteins claudin-1, occludin and ZO-1. Claudin-1 redistribution is notably apparent at the onset of the decline in TER.¹³⁴ Expression of TJ proteins occludin, claudin-4 and -5 is reduced in duodenum biopsy samples from patients with norovirus infection that may lead to epithelial barrier dysfunction.¹³⁵ HIV can also contribute to the impairment of the intestinal barrier in that the pore-forming claudin-2 is increased while the expression of the sealing claudin-1 is reduced in HIV patients.¹³⁶

Disruptions of Claudin-Mediated Intestinal Barriers

Many molecules and factors can disrupt intestinal barriers. For examples, platelet-activating factor (PAF) induces

downregulation of TJ protein claudin-1 and ZO-1 expression, shifts their localization from the cell membrane to cytoplasm and reduces TER in Caco-2 cells. Intestinal trefoil factor (ITF) can suppress PAF-induced downregulation of TJ proteins and inhibit the abnormal localization and distribution of claudin-1 and ZO-1.¹³⁷ Mycotoxin ochratoxin A is able to disrupt the barrier function of Caco-2 cells by removal of claudin-3 and -4 from the cell membrane.¹³⁸ Deoxynivalenol (DON) is a mycotoxin that often appears on cereals used for human and animal nutrition. Using porcine intestinal epithelial cell line (IPEC-J2) as a model, the integrity of cell connections is disrupted as measured by TER, and the expression of ZO-1 and claudin-3 is reduced when DON is applied to the basolateral side of the cells. Interestingly, there is no effect of DON on the IPEC-J2 when it is applied to the apical side.¹³⁹ DON also contributes to the loss of barrier function of porcine intestinal epithelial cell line (IPEC-1) through the decreased expression of claudin-4 protein, which is involved in the maintenance of the intestinal epithelial cell barrier function. DON-induced impairment of intestinal barrier is through the activation of ERK/MAPK signaling pathway. Inhibition of ERK/MAPK pathway restores the intestinal expression of claudin-4 protein.¹⁴⁰ DON increases the paracellular permeability of Caco-2 cell monolayers. This may be through its protein synthesis inhibition role by diminishing the synthesis of claudin-4.¹⁴¹ Irinotecan causes disorders in the intestinal epithelial barrier and induces bacterial translocation. Claudin-1 mRNA in the small intestine is significantly decreased and claudin-1 protein expression in both the small and large intestines is significantly reduced after application of irinotecan.¹⁴² Endocannabinoids decreases the mRNA of claudin-1 in Caco-2 cells.¹⁴³ Exposure to phenol results in decreased TER and increased paracellular flux of FITC-dextran. Delocalization of claudin-1 and ZO-1 from TJs to cytosol correlates with the permeability increase after phenol treatment.¹⁴⁴ Prolactin (PRL) can enhance intestinal absorption of Ca²⁺ and other minerals for fetal development and milk production. PRL markedly stimulates the transcellular and paracellular Ca²⁺ transport in the duodenum of pregnant and lactating rats as well as in Caco-2 cell monolayers. Claudin-15, which regulates the epithelial cation selectivity and paracellular Ca²⁺ movement, is required for this PRL-enhanced paracellular transport.¹⁴⁵ PRL is found to downregulate the expression of claudin-3 and occludin in IEC-6 crypt cells, a rat small intestine epithelial cell line.¹⁴⁶ Cofilin is an actin binding protein and its dephosphorylation corresponds to the TJ opening, TER decrease and claudin-1 dissociation from the cytoskeleton. This suggests that cofilin may serve as a target for TJ permeability regulation in epithelial cells.¹⁴⁷ Phosphatidylethanol is produced from ethanol by phospholipase D. It is accumulated after chronic ethanol exposure, which induces claudin-1 endocytosis and disrupts the claudin-1/ZO-1 association.¹⁴⁸

Oxidative stress can cause the increased colonic epithelial permeability. For example, hydrogen peroxide (H₂O₂) treatment changes the localization of claudin-4 from apical TJ to lateral membrane in Caco-2 cells.¹⁴⁹ Interestingly, incubation of T84 cells with IL-4 leads to an increased claudin-2 expression with a corresponding decrease in TER and increase in permeability

while the IFN- γ treatment has the opposite effects, leading to decreased claudin-2 and increased TER.¹⁵⁰ Gliadin is known to cause celiac disease. Treatment of gliadin on Caco-2 cells causes a reorganization of actin filaments and reduces the expression of the TJ protein occludin, claudin-3 and -4. Immunofluorescence staining of these TJ proteins reveals a decrease in plasma membrane staining and an increase in punctate, cytosolic staining after gliadin treatment.¹⁵¹ Chitosan can transiently and reversibly open the TJs between Caco-2 cells, thus enhancing the paracellular permeability. Chitosan treatment induces claudin-4 degradation and redistribution in the cells, leading to the opening of TJs. Chitosan could potentially be used to mediate the transepithelial drug delivery.¹⁵² Nanoparticle-mediated drug delivery is a fast-growing research field. Si-nanowire-coated silica microparticles alter TJ permeability and decrease the width of ZO-1 and claudin-1 at the TJ in Caco-2 cells in culture.¹⁵³ Vitamin D can suppress the expression of claudin-3 and change the permeability of the TJs, thus enhancing the paracellular Ca²⁺ transport.¹⁵⁴ Miltefosine (hexadecylphosphocholine) is the first effective oral agent for the treatment of visceral leishmaniasis. Its oral administration alters the distribution of claudin-1 and translocates it from the intercellular junctions to the cytoplasm.¹⁵⁵ Glutamine deprivation markedly decreases TER and claudin-1 expression.¹⁵⁶

Clostridium perfringens enterotoxin (CPE) is responsible for the GI symptoms of the second-most-common bacterial food-borne illness. On the other hand, CPE is also currently being explored to be used as an anti-cancer therapeutic or enhancing drug delivery agent.¹⁵⁷ C-terminal CPE (C-CPE) can bind and modulate claudin structures, thus enhancing mucosal absorption. Members of the claudin family can serve as CPE receptors and convey CPE sensitivity. Human claudin-3 and -4 are two functional CPE receptors. Recently, claudin-8 and -14 have also been shown to be able to convey the cytotoxic effect of CPE on mammalian intestines although the efficiency is 2-to-10-fold less than that of claudin-4. Specific residues responsible for modulating the CPE binding activities have been identified.¹⁵⁷ Binding of C-CPE to claudin-4 enhances jejunal absorption of dextran.¹⁵⁸ Besides CPE and C-CPE, Matsuhisa et al. have developed a C-CPE mutant that has a broad binding specificity to claudin-1, -2, -4 and -5, which could enhance the jejunal absorption of dextran. The C-CPE mutant binder exhibits a more potent jejunal absorption-enhancing effect than that of C-CPE.¹⁵⁹ The treatment of non-cytotoxic recombinant CPE variant is able to partially inhibit CPE-induced histologic damage, suggesting that non-cytotoxic recombinant CPE variants may be useful for protecting against some intestinal effects of CPE.¹⁶⁰⁻¹⁶² Tyr306 and Leu315 are key residues in C-CPE that are involved in the modulation of claudin-4.^{163,164} Takahashi et al. have reported that the C-CPE194 binds to claudin-4 and enhances mucosal absorption. Recently, they have created a mutated form of C-CPE194 by substituting asparagine at position 309 and serine at position 313 with alanine, which increases the affinity to claudin-4 by almost 10-fold as compared with C-CPE194. Deletion of 10 amino acids in the N-terminal domain of the double-alanine-substituted mutant further increases the affinity to claudin-4 by nearly 24-fold as compared with C-CPE194. Therefore, these C-CPE mutants may be a promising lead for the development of a clinical TJ modulator.¹⁶⁵

Improvements of Intestinal Barrier that Involves Claudins

A number of molecules or chemicals have been found to improve the intestinal barrier and ameliorate the alteration of claudins in pathological status and thus are used to treat the intestinal barrier dysfunction.

Moxibustion may relieve colonic epithelial barrier defect by upregulating the expression of colonic epithelial occludin, claudin-1 and ZO-1 proteins and their mRNAs in the cultivated colonic epithelial cells.¹⁶⁶ The fish oil supplement in piglets improves intestinal morphology by increasing villus height and intestinal barrier function and upregulating intestinal TJ proteins including occludin and claudin-1. Under the inflammatory condition triggered by LPS, fish oil could alleviate the LPS-induced intestinal damage.¹⁶⁷ VSL#3, a probiotic mixture, can stimulate the epithelial-derived TNF and prevents the onset of ileitis in SAMP mice by decreasing the ileal paracellular permeability through downregulation of claudin-2 and upregulation of occludin.¹⁶⁸ Butyrate improves the barrier function of GI epithelia and has a potential to treat IBD. It restores the TJs in IBD patients by downregulating the expression of claudin-1 and -2 and upregulating occludin, cingulin, ZO-1 and ZO-2.¹⁶⁹ Intestinal alkaline phosphatase (IAP) has been found to reduce the intestinal injury in a NEC rat model established by LPS administration and this effect is dose-dependent.¹⁷⁰ Bao et al. have reported that herbs-partitioned moxibustion (HPM) and mild-warm moxibustion (MWM) treatments significantly enhances the expression of occludin, claudin-1 and ZO-1 and improves the microstructure of the colonic epithelial barrier in a CD rat model established by TNBS treatment.¹⁷¹ Recently, Moran et al. have found that Glucagon-like peptide-2 (GLP-2), an intestinotrophic enteroendocrine peptide, can increase the TER by upregulating the protein expression of occludin and ZO-1 in Caco-2 cells.¹⁷² Using the HT-29/B6 cells as a model, Hering et al. assess the barrier-protective effects of TGF- β (isoforms 1–3) on intestines. TGF- β increases the claudin-4 expression at the transcriptional level by binding to the promoter of claudin-4.¹⁷³ Nicotine treatment on Caco-2 cell monolayers significantly improves TJ integrity as measured by TER and significantly upregulates occludin and claudin-1.¹⁷⁴ Kaempferol, a natural flavonoid present in fruits, vegetables and teas, provides beneficial effects for human health. Kaempferol enhances the TJ integrity in Caco-2 cells. The protein levels of claudin-1, -3 and -4 on the cell membrane are increased during the first 6 or 12 h after kaempferol administration. Confocal fluorescent microscopy reveals higher immunofluorescence intensities for claudin-3 at the intercellular junctions than the untreated controls after kaempferol treatment for 6 h.¹⁷⁵ Flavonoid quercetin enhances epithelial barrier function and increases claudin-4 expression in Caco-2 cells.¹⁷⁶ Methionine-restricted (MR) diet in rats results in the improved colonic epithelial barrier function compared with the rats on the control diet by altering TJ protein composition. MR diet increases TER with concomitant decrease in paracellular permeability. RT-PCR and western blot analysis show an 80% increase in claudin-3 mRNA and 5-fold increase in claudin-3 protein level.¹⁷⁷ IL-15 is able to enhance the TJ formation in intestinal epithelial cells. The recruitment of claudin-1 and

Table 4. Factors affecting claudin expression and intestinal barrier

Factors disrupting intestinal barrier	Claudin(s)	References	Factors improving intestinal barrier	Claudin(s)	References
platelet-activating factor	1 ↓	137	intestinal trefoil factor	1 ↑	137
mycotoxin ochratoxin A	3 ↓, 4 ↓	138	moxibustion	1 ↑	166
deoxynivalenol	3 ↓, 4 ↓	139, 140, 141	fish oil	1 ↑	167
irinotecan	1 ↓	142	VSL#3	2 ↑	168
endocannabinoids	1 ↓	143	butyrate	1 ↓, 2 ↓	169
prolactin	3 ↓	146	intestinal alkaline phosphatase	1 ↑, 3 ↓	170
interleukin 4	2 ↑	150	TGF-β	4 ↑	173
gliadin	3 ↓, 4 ↓	151	nicotine	1 ↑	174
chitosan	4 ↓	152	kaempferol	1 ↑, 3 ↑, 4 ↑	175
vitamin D	3 ↓	154	flavonoid quercetin	4 ↑	176
glutamine	1 ↓	156	interleukin 17	1 ↑, 2 ↑	179

-2 to the cell membrane are enhanced by IL-15 in the presence of IL-2Rβ subunit, a subunit of IL-15 receptor in T84 cells.¹⁷⁸ IL-17 upregulates claudin-1 and -2 at the transcriptional level, which induces the formation of TJs in T84 cells.¹⁷⁹ As a summary, the above several major factors affecting the intestinal barrier by either disrupting or improving TJ structure and function have been listed in Table 4.

Conclusion

TJs in enterocytes separate the intestinal lumen from the underlying tissues, thus maintaining the homeostasis of intestines. Claudins are essential components of TJs regulating paracellular solute transport. Claudins can alter or be altered by a number of signaling molecules/pathways. Abnormal expression and/or mis-localization of claudins are associated with various pathophysiological conditions of intestines. Studying the molecules/

chemical agents that regulate the claudins, either by enhancing or ameliorating the intestinal permeability, can provide an opportunity for exploring prospective approaches for treating the intestinal diseases or delivering drugs. More studies are needed in order to understand whether the changes in claudins are the causes or the consequences of the intestinal diseases and the underlying mechanisms related to the intestine disorders.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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