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Intestinal epithelial claudins: expression and regulation in homeostasis and inflammation

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Abstract

The intestinal epithelium forms a highly dynamic and selective barrier that controls absorption of fluid and solutes while restricting pathogen access to underlying tissues. Barrier properties are achieved by intercellular junctions that include an apical tight junction (TJ) and subjacent adherens junction and desmosomes. The TJ tetraspan claudin proteins form pores between epithelial cells to control paracellular fluid and ion movement. In addition to regulation of barrier function, claudin family members control epithelial homeostasis and are expressed in a spatiotemporal manner in the intestinal and crypt-luminal axis. This delicate balance of physiologic differential claudin protein expression is altered during mucosal inflammation. Inflammatory mediators influence transcriptional regulation, as well as endocytic trafficking, targeting, and retention of claudins in the TJ. Increased expression of intestinal epithelial claudin-1, -2, and -18, with downregulation of claudin-3, -4, -5, -7, -8, and -12, has been observed in intestinal inflammatory disorders. Such changes in claudin proteins modify the epithelial barrier function in addition to influencing epithelial and mucosal homeostasis. An improved understanding of the regulatory mechanisms that control epithelial claudin proteins will provide strategies to strengthen the epithelial barrier function and restore mucosal homeostasis in inflammatory disorders.

Keywords

epithelium; intestine; mucosal barrier; tight junction; claudins; inflammation

Introduction

Epithelial cells form selective barriers that interface distinct internal and external environments. While forming a protective barrier to pathogen access, the epithelium controls select movement of ions, fluid, and solutes. Epithelial barrier function is achieved by a series of intercellular junctions that include an apical tight junction (TJ) and subjacent adherens junction, which are collectively referred to as the apical junctional complex (AJC). Desmosomes and gap junctions, which reside below the AJC, mediate intercellular adhesion and cross talk between adjacent epithelial cells.[1,2] In the intestine, a single layer of epithelial cells is organized in densely packed invaginations referred to as crypts (Fig. 1A). Small

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intestinal epithelial cells (IECs) also line luminal protrusions or villi. The intestinal epithelium is highly dynamic and is actively turned over as progenitor stem cells in the crypt proliferate, differentiate in the crypt–luminal axis, and are shed in a regulated manner into the gut lumen.[3] It is remarkable that epithelial barrier properties are not only maintained but are also modified as the enterocytes navigate the crypt-luminal axis. Here, we largely focus on the complexity and function of intestinal epithelial claudins and touch upon recent concepts on how claudins are regulated during epithelial differentiation and in response to mucosal inflammation.

Tight junctions

Epithelial TJs reside at the junction between the apical and basolateral plasma membranes and regulate epithelial polarity and vectorial movement of solutes and fluids in the intercellular space (paracellular pathway).[4–6] Dynamics of the paracellular pathway are intimately linked with transcellular transport mechanisms that maintain homeostasis across polarized cells.[7,8] Such coordination between paracellular and transcellular movement of solutes also serves as a mechanism to save energy while ensuring polarized fluid and solute movement across polarized cells.[8,9] In addition to these functions, TJ proteins actively participate in mediating communication between intercellular contacts and cellular homeostasis encompassing cellular proliferation, differentiation, and migration. TJs are visualized as areas of close membrane apposition between cells by transmission electron microscopy and as anastomosing multistrand structures by freeze–fracture electron microscopy.[5] The protein-containing strands span the extracellular space between adjoining cells, and properties of the TJ seal have been determined by protein composition of strands and their ultimate organization within the junction. Thus, the TJ strand organization varies in epithelial cells with different permeability properties.[10,11] Maintenance of TJ strands requires association of proteins that constitute the strands with submembranous cytoplasmic plaque proteins that serve as scaffolds to link TJ strands to the apical actin cytoskeleton. The contractile properties of this perijunctional actin actoskeleton plays an important role in controlling the physiologic TJ function.

The three main families of TJ transmembrane proteins are claudins, TJ-associated MARVEL proteins (TAMPs), and the cortical thymocyte marker in *Xenopus* (CTX).[12] Occludin, MarvelD3, and tricellulin constitute the TAMP family of proteins. The epithelial CTX family includes JAM-A (junctional adhesion molecule), CAR (coxsackie virus and adenovirus receptor) and CLMP (CXADR-like membrane protein). While the TAMP family of proteins has been implicated in influencing barrier function, proteins such as occludin have important signaling properties that regulate epithelial homeostasis.[13] The CTX family protein JAM-A regulates epithelial TJ barrier function by influencing claudin protein expression and actin–myosin dynamics, thereby influencing properties of both the TJ pore and leak paracellular pathways. Permeability properties are further modified at epithelial tricellular contacts that contain additional transmembrane proteins, such as tricellulin and LSR (lipolysis-stimulated lipoprotein receptor).[14]

Transmembrane proteins associate with subjacent plaque proteins that provide a link to underlying actin filaments. These associations are dynamically regulated by myosin II and

affiliated proteins to control TJ function.[15] In addition to structural plaque proteins that link transmembrane proteins to the actin cytoskeleton, the TJ submembranous plaque comprises a plethora of regulatory proteins, including small GTPases, kinases/phosphatases, and transcription factors. In fact, over 80 proteins have been identified in the TJ, suggesting that the associated proteins function as signaling hubs that orchestrate a number of cellular events, such as epithelial polarity, homeostasis, and barrier function.[16–19]

The TJ is a dynamic structure that is actively remodeled during the life span of the epithelium.[20–22] Furthermore, the TJ protein composition is modified in response to a number of cues in the intestinal crypt-luminal axis. Such delicate control mechanisms are perturbed in pathologic states, such as inflammation and cancer, that influence epithelial homeostasis and barrier properties.[23–26] The paracellular movement of molecules has been suggested to occur by “pore” or “leak” pathways. Pores between cells are structurally formed by claudins that serve as a low-capacity, ion-charge, and size-selective pathway. While the molecular mechanism that form the basis of the leak pathway is less well understood, it has been proposed to be regulated by changes in TJ strand organization, including strand breaks and transient paracellular gaps that allow large molecule non-selective flux.[27–31] This latter leak pathway is regulated by transmembrane proteins, as well as by scaffold protein and tension of the perijunctional actin myosin cytoskeleton.

Claudins

The tetraspan claudin family of proteins includes 26 family members in humans. Ultrastructurally, claudins reside in TJ strands and serve to control the charge and size selective properties of the paracellular space, thereby regulating barrier properties. Claudins have been proposed to function as “tight” or sealing claudins or as “leaky” pore-forming claudins. The paracellular pore properties have been attributed to claudin amino acid composition in the first extracellular loop (EC1).[32,33] Pore- or channel-forming claudins have selectivity for charged ions and water. Cysteine residues in the EC1 enhance the stability of the protein, while the smaller second extracellular loop (EC2) mediates claudin–claudin interactions within a cell (*cis*) and in adjoining cells (*trans*)[27,34](Fig. 1B). Tight claudins include claudin-1, -3, -4, -5, -6, -8, -12, -18, and -19,[35–41] while leaky claudin-2 and claudin-15 contribute to increased paracellular permeability to sodium and water.[42–44] While these distinctions have been proposed, the overall function of a claudin is dependent on complement of other claudins expressed within a TJ. The recently solved claudin-15 crystal structure has provided significant advances in understanding of its higher-ordered organization in TJs and shed light on pore characteristics that mediate ion and fluid movement in the paracellular space.[45]

Claudin-1 is widely expressed in the intestinal epithelium, is known by its barrier-forming abilities, and has been proposed to have an important role for TJ integrity.[35,46] Claudin-3 expression is higher in the colon than in the rest of the gut[47] and has been classified as a barrier protein.[48] However, converse effects of claudin-3 on barrier function have been reported in lung alveolar epithelia.[49] In an analogous manner, the role of claudin-4 in controlling barrier function is dependent on the background claudin family members within a junction. *In vitro* studies have identified barrier-forming abilities[50] of claudin-4 in colonic

epithelial cells.[51] Claudin-5 enhances barrier function in model IECs[37] and plays a pivotal role in promoting barrier properties of endothelial cells in the blood–brain barrier,[52] while its importance in controlling *in vivo* intestinal epithelial barrier function and homeostasis is not clear. There is contrasting evidence showing that claudin-7 can function as an anion pore or cation barrier in porcine and dog kidney cells, which is most likely dependent on the background of other claudins that are expressed within the TJ.[38–40] The specific contribution of claudin-7 in controlling the intestinal epithelial paracellular pathway is, however, not well understand. Epithelial-specific downregulation of claudin-7 increases paracellular movement of small organic solutes, suggesting that it contributes to the intestinal barrier of epithelial cells.[53] Claudin-8 function has been largely explored in the kidney,[54] where aldosterone-induced increase in claudin-8 expression has been linked to increased barrier function and decreased sodium movement into the apical compartment of epithelial cells.[55–58] Claudin-2[59–61] and claudin-15[44,62] regulate paracelullar transport of Na^+ and water in the intestine. These pore- or channel-forming claudins are widely expressed in the proliferative compartment of the colonic crypt epithelium. Mice with intestinal epithelial loss of claudin-15 have low intraluminal sodium that influences sodium-coupled glucose absorption via the apical sodium glucose co-transporter SGLT.[44]

The majority of claudins, with a few exceptions, such as claudin-23, contain a PDZ (PSD95, Dlg1, and ZO-1) binding motif that associates with submembrane plaque proteins containing PDZ domains and regulatory molecules, which serve to organize and control TJ structure and ultimately epithelial barrier function.[63–66] The most extensively studied cytoplasmic plaque PDZ domain–containing proteins are the zonula occludens (ZO) proteins, which includes ZO-1 and ZO-2. However, the mechanisms that govern preferential association of family members with specific plaque proteins remain less well understood. Freeze–fracture ultrastructural analysis has revealed that claudins reside and organize TJ strands between cells.[62] A recent study using super-resolution live-cell imaging elegantly demonstrated the contribution of ZO-1 in stabilizing claudin strands with the actin cytoskeleton.[67] Such TJ protein–actin cytoskeleton affiliations control physiologic epithelial barrier properties and are targeted in pathologic states associated with barrier compromise. Posttranslational modifications of claudins have been suggested to influence their association with scaffold proteins in the TJ.[68,69]

While the plaque protein play an important role in controlling TJ transmembrane protein organization, claudin proteins have an innate ability to self-organize/oligomerize within TJs. Claudin proteins can interact in *cis* and in *trans*, and not all claudins are pair compatible. Within a TJ, the complement of claudins ultimately determines the properties of the paracellular pore pathway in epithelial and endothelial cells.[70,71] While TJ plaque proteins play important roles in stabilizing and regulating transmembrane claudins, claudin proteins can be targeted to the TJ independent of these associations. *In vitro*–expressed claudins-1 and -5 lacking the PDZ binding motif localize to TJs.[66] However, loss of the entire C-terminal juxtamembrane sequence prevents their association with TJ of epithelial and endothelial cells, suggesting that the amino acid composition in this region contributes to claudin protein–mediated barrier function.[66] Interestingly, within a TJ, differential claudin protein associations have been reported in strands that reside in the basal versus apical aspects of the junction. Such organization has been postulated to occur in response to shear

stress to which the junction is exposed.[72] While such variability in claudin-containing strands of intestinal epithelial TJ have not been explored, one can envision that such organization exists in IECs that are continuously exposed to physical forces associated with epithelial turnover and gut motility.

Amino acids in the claudin X-terminus undergo posttranslational modifications (phosphorylation, palmitoylation, and sumoylation)[63–65] that have been proposed to control their subcellular localization, protein levels, and stability.[66] Such modification could potentially play an important role in claudin isoform switching in response to environmental cues.

Spatiotemporal expression of intestinal epithelial claudin proteins

The composition of epithelial claudins not only varies spatially along the length of the gastrointestinal tract but also in the crypt-luminal axis as epithelial cells transition from a proliferative to differentiated phenotype (Fig. 2). It is remarkable that such claudin remodeling occurs within a few days during the life span of the intestinal epithelium that maintains and yet modifies its barrier properties.[73] However, mechanisms that govern such physiologic remodeling of TJ claudins in differentiating IECs are not well understood.

A number of studies have investigated the spatiotemporal distribution of claudin proteins in the intestine (Fig. 2A). In humans, claudin-1, -2, -7, -12, and -15 mRNA was detected along the entire intestinal tract axis. While abundant claudin-5 expression was identified in the duodenum, claudin -3, -4, -7, and -8 exhibited increased expression in the distal colonic epithelium.[74] In the adult mouse and rat intestine, reverse transcriptase polymerase chain reaction (RT-PCR) analysis identified expression of claudin-1, -2, -3, -4, -5 -7, -8, -9, -10, -11, -12, 13, -14, -15, -17, and -18, while claudin-6, -16, -19, -22, and -24 were not detected. Claudin-2, -3, -7, and -15 mRNA are the most highly expressed in the gut.[75–77]

Increasing claudin-8 mRNA expression has been reported in distal versus proximal IECs.[75,76] The converse has been reported for claudin-15. Peak claudin-2, -5, -7, and -10 mRNA expression has been noted in TJs of epithelial cells in the ileocecal junction. In contrast, intestinal epithelial claudin-18 is expressed in the esophagus, stomach, duodenum, and jejunum,[75,78,79] while claudin-13 mRNA was identified in the large intestine/colon, with increased expression in luminal epithelial cells.[76]

In the crypt-luminal axis, mRNA expression of pore-forming claudin-2, -10, and -15 is restricted in the crypt base, while barrier-forming claudin-3 and claudin-4 were observed to be exclusively expressed in luminal epithelial cells[75–77] (Fig. 2B and 2C). In a recent study, we mapped the spatial distribution of claudin mRNA expression in colonic epithelial cells after microdissection of crypt versus luminal epithelial cells. Claudin-3, -4, -7, - 8, and -23 were detected in luminal epithelial cells.[80] The contribution of claudin-23 in controlling intestinal epithelial barrier function, however, remains unexplored. Given its spatial expression in luminal IECs,[80] claudin-23 likely contributes to the barrier-forming abilities of other claudin protein family members expressed at these sites. In contrast, uniform

distribution of claudins1 and claudin-12 was observed in the entire colonic crypt-luminal axis.[47,81]

The specific organization of claudins in adult IECs differs from that in the developing intestine. In mice, mRNA expression of intestinal epithelial claudin-1, -2, -5, and -8 is lower at 1 and 90 days after birth, while claudin-19 expression is exclusively detected 1–14 days postpartum. At birth, the pore-forming claudin-2 is expressed in the entire intestinal crypt-luminal axis and becomes restricted to the crypt base in the colon 90 days after birth. Increasing levels of intestinal epithelial claudin-3, -4, -7, and -15 have been identified in the maturing intestine after birth.[75]

Besides the above spatial organization, at the cellular level claudin proteins can be either targeted exclusively to the TJ or also reside in the lateral membrane of polarized epithelial cells (Fig. 2D). While claudin- 2, -8, -10, -12, -15, and -18 proteins have restricted TJ localization, claudin-1, -3, -4, -5, and -7 are also targeted to the lateral plasma membrane (Fig. 2C).[82] Lateral expression of claudin-7 in IECs has been proposed to have signaling properties that influence maintenance of extracellular matrix interactions, homeostasis,[83] and differentiation.[84] The functional role of lateral membrane–associated claudins is not well understood. This pool of claudins could function as a reservoir for TJ-associated claudins during dynamic remodeling of the junctional protein complex. This possibility has been proposed in studies that have utilized fluorescence recovery after photobleaching (FRAP) to study remodeling of intestinal epithelial junctional claudin proteins.[21,85] Furthermore, lateral membrane–associated claudin proteins could also have signaling function that controls epithelial homeostasis.

In addition to their pivotal role in controlling barrier function, claudins regulate epithelial homeostasis that encompasses proliferation and differentiation of cells. While many of the above studies have mapped the topographical distribution of claudin proteins in intestinal TJs, the mechanisms by which claudin remodeling is achieved remain less well understood. It is, however, clear that claudin organization in the intestinal tract, as well as in the crypt-luminal axis, responds to intracellular and extracellular cues that control physiologic properties of the epithelia barrier. Dysregulation of claudin proteins has been observed in inflammatory states associated with a leaky epithelial barrier, as well as in neoplastic states.[23,25,26,86–91] In these scenarios, claudin proteins have been proposed to actively contribute to disease pathogenesis.

Transcriptional regulation of claudins in intestinal epithelial cells

Claudin remodeling is orchestrated by transcriptional regulation, posttranslational modifications, intracellular trafficking, and stability in the plasma membrane. The differential expression of claudin proteins in the crypt-luminal axis has been linked to epithelial proliferation and differentiation programs.

Limited information is available on the transcriptional programs that control CLDN genes. β-Catenin–TCF/LEF, CDX2, and HNFα transcription factors (TFs) have been implicated in regulating the expression of claudin family members in the intestine. The β-catenin–

TCF/LEF signaling pathway plays an important role in controlling intestinal crypt epithelial cell proliferation, and previous studies have identified a link between β-catenin signaling and claudin-1 expression.[92] The claudin-1 promoter contains TCF/LEF-binding sites, and decreased claudin-1 expression has been reported after downregulation of β-catenin. Furthermore, increased claudin-1 expression is associated with enhanced cellular proliferation.[93] The transcription factor FOXO4 is involved in oxidative stress and insulin signaling, longevity, cell cycle progression, and apoptosis. Downregulation of claudin-1 was noted in epithelial cells after FOXO4 depletion. These observations suggest that claudin-1 expression is linked to intestinal epithelial cell proliferation and plays a role in the regulation of epithelial homeostasis. The intestinal epithelium resides in a low-oxygen environment, and the family of hypoxia-inducible factors (HIFs) control epithelial maintenance and repair.[94] HIF-1β depletion in IECs results in barrier defects. Furthermore, hypoxia-response elements have been identified in the claudin-1 promoter, and IEC HIF-1β knockdown decreases claudin-1 mRNA.[46] These observations support a unique relationship among HIF TFs, claudin-1, and intestinal epithelial barrier function that is maintained in a relatively hypoxic environment.

The TFs CDX-1 and CDX-2 have been reported to regulate intestinal epithelial development and physiologic epithelial homeostasis.[95] The claudin-1 and claudin-2 promoters contain several CDX-2–binding sites, and CDX-2 promoter binding was demonstrated using chromatin immunoprecipitation assays.[96] Overexpression of CDX-2 in cell lines SW480 and HCT116 resulted in increased claudin-1 mRNA. Additionally, coexpression of CDX-2 and active β-catenin had synergistic effects in increasing claudin-1 mRNA. Both CDX-1 and CDX-2 activate the claudin-2 promoter in the Caco-2 model (a human IEC line). Interestingly, hepatocyte nuclear factor (HNF) 1α potentiates CDX2-mediated transactivation of claudin-2. This effect is further enhanced in the presence of the TF GATA4.[97–100] These findings not only support an interplay between these transcription factors that serve to control claudin mRNA expression but also highlight the complexity of claudin protein regulation in the intestine.

HNF4α is a member of the steroid-receptor family of TFs that localizes exclusively to the nucleus and has been implicated in the regulation of intestinal epithelial barrier function in mice.[101] HNF4α binds the claudin-7 and claudin-15 promoters, and in a recent study we observed a relationship between HNF4α and claudin-7 expression in differentiating IECs.[84] A gradient of HNF4α expression in the intestinal epithelial crypt-luminal axis was identified, with increased expression in luminal epithelial cells that also express claudin-4, -7, and -23 mRNA. In contrast to HNF4α, the TFs KLF4 and HOPX were restricted to crypt-base IECs that express claudin-2, -5, and -15.[80]

Intracellular trafficking of claudin proteins

Intracellular trafficking of claudin proteins contributes to the remodeling of TJs in physiologic and pathologic states. Internalization of claudin-based TJ strands has been observed in the rat intestine and in the human fetal hindgut.[102,103] Such protein trafficking contributes to the rapid changes in cell adhesion, migration, and extrusion of apoptotic cells in the intestine. Claudin protein endocytosis has been reported during the epithelial-to-

mesenchymal transition and in response to inflammatory mediators, oxidative stress, and ischemic injury. Depending on the stimulus, select junctional proteins, including claudins, are internalized from the cell surface through a number of mechanisms that differentially involve clathrin, caveolin, and micropinocytosis pathways.[104] Internalized TJ proteins are delivered to early endosomes, followed by their trafficking to recycling endosomes to be targeted back to the TJ or into late endosomes for degradation.

Biochemically, TJ proteins are identified in an immobile stable pool of proteins integrated into the TJ and a mobile unstable pool of proteins peripheral to the TJ.[85] Despite the stability of claudin-based TJs in confluent epithelia, these structures undergo steady-state remodeling that involves constant endocytosis and recycling of different claudin proteins.[105,106] Interestingly claudin family members exhibit differential dynamics within TJs. For example, claudin-1, -2, and -3 are readily internalized and recycled, whereas claudin-4 shows negligible endocytosis in confluent epithelial monolayers. Such endocytosis/recycling of TJ proteins most likely contributes to homeostatic movements and/or steady-state remodeling of epithelial cell–cell contacts under physiological conditions.

Lessons from mice with genetically modified intestinal epithelial claudins

A number of mice with genetically modified expression of claudins have been generated to investigate the functional role of specific claudin family members in controlling epithelial barrier function. Transgenic mice that overexpress claudin-1 (TG) in IECs exhibit upregulation of MMP-9 and ERK that is associated with Notch activation.[107] Previous studies have demonstrated a suppressive role of Notch signaling in intestinal epithelial differentiation that is evidenced by decreased goblet cells and mucin-2 (Muc-2) synthesis (Fig. 3A).[108] Such observations in claudin-1 TG mice provide insight into a link between claudin-1 and Notch signaling that could serve to control intestinal epithelial homeostasis, differentiation, and mucosal barrier function.

Claudin-2 regulates paracellular movement of Na^+, Ca^{2+}, and water in the intestine.[42,44,109] Deletion of the claudin-2 gene in mice decreases the transepithelial conductance and paracellular permeability for Na^+.[109] Specific overexpression of claudin-2 in the intestinal epithelia results in increased length of colonic crypts and an overall enlargement of the intestine that is associated with increased paracellular permeability to the Na^+ ion and solute FITC–dextran. Interestingly, claudin-2 also promotes colonic epithelial cell proliferation (Fig. 3B).[110,111] This was previously shown in a human lung adenocarcinoma cell line, where claudin-2 nuclear distribution increases epithelial cell proliferation by promoting translocation of ZONAB into the nucleus.[112]

Claudin-7 global knockout mice have increased intestinal mucosal inflammation during postnatal development that is associated with enhanced epithelial cell proliferation and apoptosis. Additionally, deletion of claudin-7 was associated with a 120-fold increase in intestinal metalloproteinase-3 (MMP-3) mRNA, which was implicated in promoting extracellular matrix degradation and the mucosal immune response.[83] In another study,

intestinal epithelial–specific knockout of claudin-7 resulted in increased paracellular permeability to solutes (457 and 4000 Da) (Fig. 3C).[53]

Claudin-15 KO mice exhibit megaintestine, which is associated with nutritional defects as a result of impaired intestinal Na^+ and K^+ paracellular permeability and decreased glucose absorption.[44,113] The molecular basis of these phenotypes is likely related to claudin-15 regulation of epithelial homeostasis (Fig. 3D).[113] In keeping with this notion, claudin-15 loss due to a mutation in the transcription factor TCF2 was associated with abnormal intestinal development.[114] Furthermore claudin-2 and-15 double-knockout mice have severe malnutrition as a result of defects in intestinal epithelial absorption of glucose, amino acids, and lipids, leading to death after birth (Fig. 3E).[115]

Inflammation and claudin expression in the intestine

Mucosal inflammation as observed in acute colitis/enteritis and inflammatory bowel disease compromises the epithelial barrier, resulting in the exposure of lamina propria tissue compartments to luminal antigens and microbes that further contribute to the inflammatory response and barrier defects.[116,117] The barrier damage occurs in response to inflammatory mediators as well as trafficking of immune cells to sites of inflammation. Several studies have reported differential effects of such inflammatory mediators on TJ proteins (Fig. 4A). Increased expression of claudin-1, -2, and -18[25,86,118–120] and downregulation of claudin-3, -4, and -7 was reported in ulcerative colitis.[25,118] In Crohn's disease, upregulation of claudin-1 and claudin-2 and decreased expression of intestinal epithelial claudin-3, -5, -8, and -12 were observed[25,26,74,86,121] (Table 1). In response to inflammation, altered claudin protein profiles in the TJ are associated with perturbed paracellular movement of fluid and solutes, which is reflected in overall change in epithelial barrier function. Replacement of barrier-forming claudins with pore/channel-forming claudins ultimately influences ion and fluid movement across cells, which is clinically reflected in disease symptoms, including diarrhea. However, it is important to take into account that changes in the level of a claudin or complement of claudins during an inflammatory response can not only result in change to the barrier properties but can also function in the protective response to host defense. An example of this scenario is claudin-2. In the physiologic state, claudin-2 expression is restricted to proliferative colonic crypt base epithelial cells. During mucosal inflammation, claudin-2 expression is upregulated in cells and its expression extends beyond the crypt-base proliferative cells in the colon. *In vitro* analyses have identified upregulation of claudin-2 in response to T_H1 and T_H2 proinflammatory cytokine exposure.[122] Transcriptional regulation, intracellular trafficking, and retention of claudin-2 in the TJ influences overall claudin-2 protein levels.[42,123,124] The molecular basis of the spatial claudin-2 expression in the crypt-luminal axis, however, is not well understood. Increase in claudin-2 has been observed in the context of decreased claudin-8[26,54] in Crohn's disease (Table 1). While claudin-2 has been linked to increased paracellular permeability, it also has protective roles, as has been observed in an experimental model of colitis.[125] While the direct mechanistic basis of claudin-2–mediated barrier protection is not understood, indirect mechanisms by which claudin-2 exerts such a protective effect include enhanced synthesis of TGF-β, which suppresses the immune response through inhibition of the inflammatory mediators, such as NF-ƙB and STAT-3.[125] Mice lacking STAT6 in the intestinal epithelium are less sensitive to

oxazolone-induced colitis, which is also associated with decreased claudin-2 expression and generation of T_H2 cytokines in the colon and inflammatory mediators.[126]

Increased claudin-18 expression has been noted in the intestinal mucosa of ulcerative colitis patients and in mice with trinitrobenzenesulfonic acid (TNBS) colitis.[127] Since claudin-18 is predominantly expressed in the stomach, its induction (along with expression of some other markers of gastric epithelium) suggests localized shifts in intestinal stem cell differentiation toward the gastric program in ulcerative colitis patients.[127,128] The increase of claudin-18 was also recently demonstrated in epithelial organoid cultures from ulcerative colitis patients[128] (Table 1).

Biopsies from individuals with ulcerative colitis have increased epithelial claudin-1 in actively inflamed intestinal mucosa that is accompanied by elevated levels of cleaved Notch and suppression of Muc-2.[107] Deletion of the Muc-2 gene in mice increased sensitivity to dextran sodium sulfate (DSS) colitis and delayed tissue repair. However, *HIF1β* knockout mice with TNBS colitis have decreased epithelial claudin-1 levels, and deletion of the HIF1β gene in Caco-2 cells decreases claudin-1 expression, which is associated with barrier compromise.[46] The above studies suggest that inflammation-mediated changes in claudin-1 are context dependent and vary with the nature of injury, time of onset, and duration of the inflammatory response. Changes in the widely expressed claudin-3 in the intestine have been observed in the mucosal tissue of inflammatory bowel disease patients,[25] and claudin-7 is decreased in the intestinal epithelium of individuals with ulcerative colitis.[83,118]

A number of *in vitro* and *in vivo* studies have addressed mechanisms by which inflammatory mediators (TNF-α, IFNγ, IL-1, IL-4 IL-6, IL-13, IL-17) modify the claudin composition of TJs in different cell lines[100,119,129–134] (Table 2). In response to inflammation, differential changes in expression of select intestinal epithelial claudins influence the overall balance of TJ claudins and, ultimately, barrier function. We recently observed that increased expression of intestinal epithelial claudin-2 in response to IFNγ signaling influences the dynamics of the barrier-forming claudin-4 that are mediated by a competition of claudin-2 and claudin-4 for residence within the epithelial TJ, thereby contributing to barrier compromise.[124] The proinflammatory cytokines IFNγ and TNF-α promote endocytosis of select TJ transmembrane proteins, including claudin-1 and claudin-4, from the plasma membrane. The underlying mechanisms that mediate this effect are dependent on the stimulus that induces junctional remodeling. While IFNγ promotes endocytosis of TJ transmembrane proteins, including claudin-1 and occludin, TNF-α exposure promotes endocytosis of occludin via a caveolar-mediated mechanism.[135,136] Such endocytic events are further orchestrated by restructuring of the perijunction actin–myosin II. In response to proinflammatory cytokine (IFNγ, TNF-α, IL13, IL-1β) signaling proteins that converge to influence perijunctional actin myosin II dynamics, TJ transmembrane protein restructuring and barrier changes include phosphorylation and activation of myosin light-chain Rho GTPase, with downstream activation of Rho-associated kinase and myosin light-chain kinase.[137] These signaling mediators maintain balanced actin–myosin contraction that is important in controlling overall TJ-mediated barrier function (Fig. 4B).[22] Additionally, *in vitro* studies suggest that TNF-α signaling also influences proteins involved in controlling epithelial polarity (atypical PKC–PAR6–PAR3) and the TJ plaque protein ZO1.[138]

Conclusions

Dynamic physiologic claudin protein regulation along the intestine, as well as in the crypt-luminal axis, plays a vital role in controlling barrier function and mucosal homeostasis. While the mechanisms that control spatial claudin expression are incompletely understood, epithelial transcriptional control and intracellular trafficking programs linked to cell proliferation and differentiation have been proposed to orchestrate such regional expression programs. Such precise organization of claudin proteins is perturbed in response to mucosal inflammation that compromises the intestinal epithelial barrier function, which further perpetuates the inflammatory response. In addition to playing a pivotal role in controlling epithelial barrier function, recent studies have shed light on claudin protein regulation of cellular programs that govern epithelial homeostasis. Future studies aimed at identifying spatial control of claudin proteins in the gut will not only be important in understanding the pathogenesis of diseases associated with the leaky epithelial barrier but will also provide direction for developing therapies to enhance epithelial barrier function and inhibit mucosal inflammatory responses.

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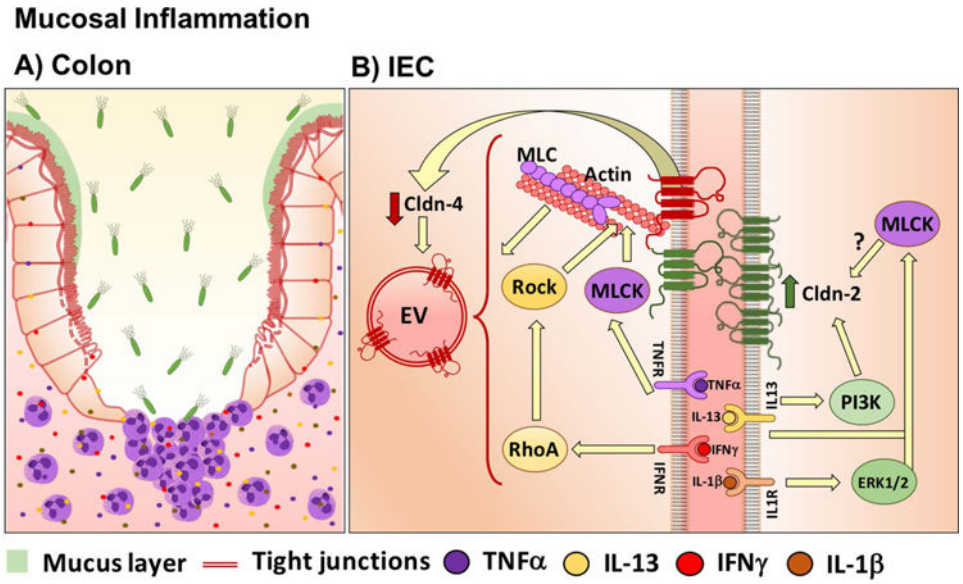

Figure 1.

Claudin functional domains and key binding partners that mediate intestinal epithelial homeostasis. (A) In the colon, a single layer of epithelial cells is organized in crypts. (B) Schematic representation of claudin functional domains and their key binding partners in the tight junction. IEC, intestinal epithelial cells; TJ, tight junction; EC, extracellular loop; PTMr, posttranslational modifications region; PDZbm, PDZ binding motif.

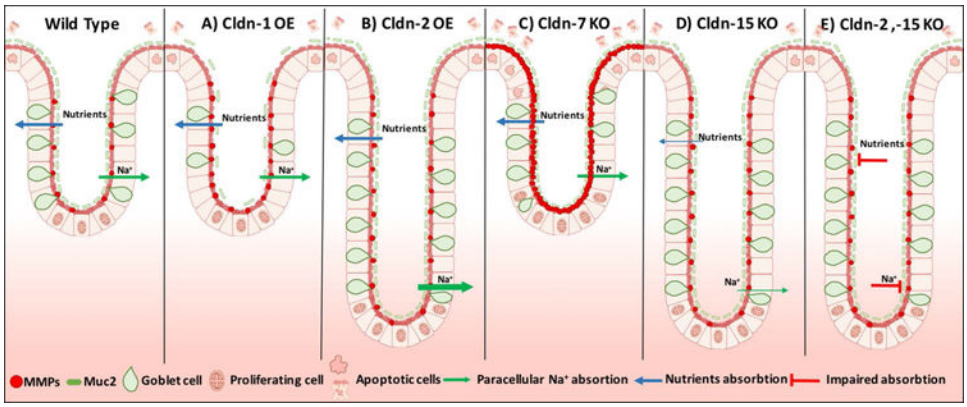

Figure 2.

Claudin expression along the intestinal tract. Schematic representation of reported claudin expression. (A) Along the intestinal tract. (B and C) Crypt-luminal axis of small and large intestine. D) Membrane distribution in the TJ or in the lateral membrane of intestinal epithelial cells (C). Cldn, claudin; IECs, intestinal epithelial cells.

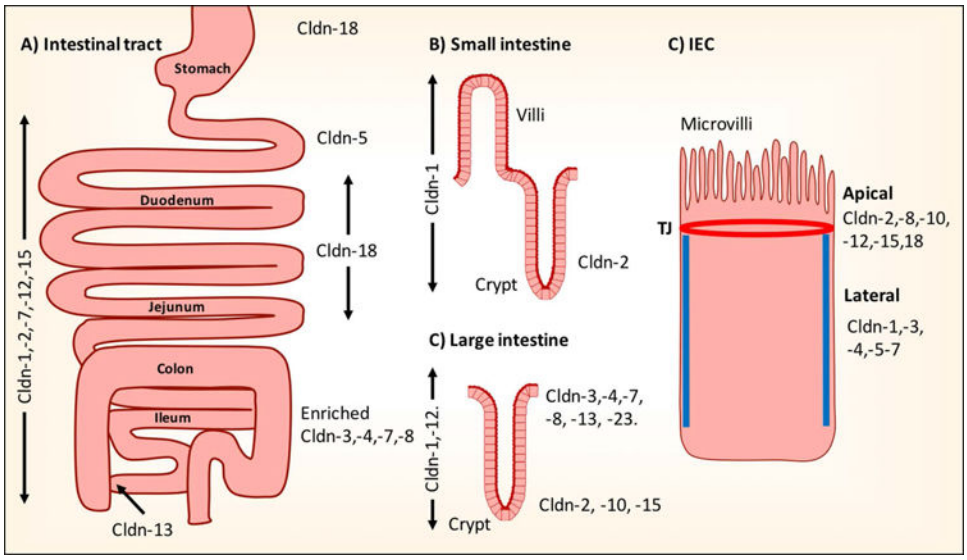

Figure 3.

Phenotypic findings in the intestinal epithelial crypts of claudin transgenic mice. OE, overexpression; KO, knockout. Thickness of the arrows represent level of absorption.

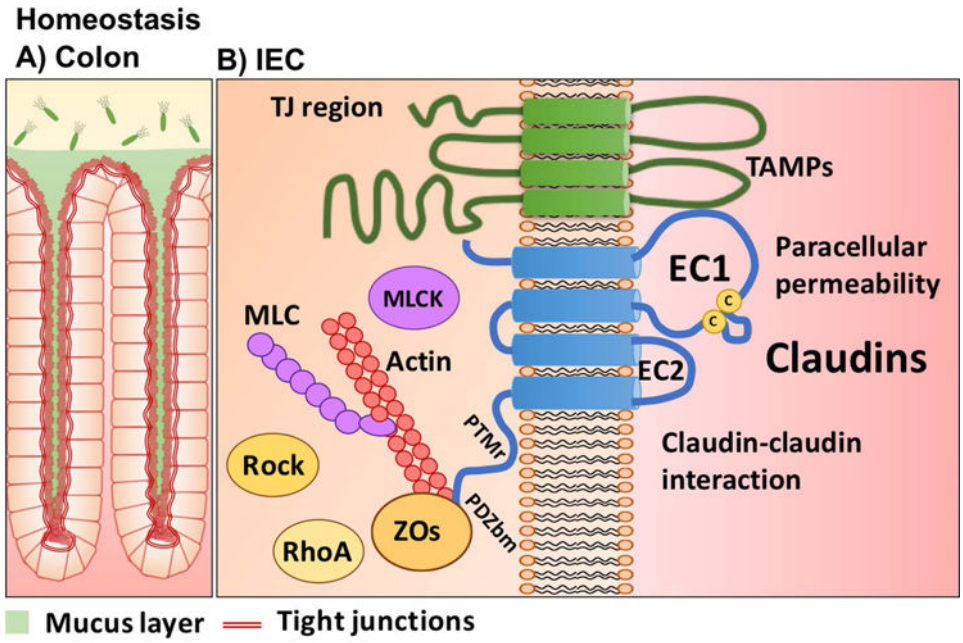

Figure 4.

Influence of inflammatory mediators on intestinal claudin proteins. Cldn, claudin; EV, endocytic vesicle

Table 1

Claudin expression in the colon during IBD (Crohn's disease and ulcerative colitis) and experimental colitis murine models.

Claudin	Function/permeability properties	Experimental colitis in mice	IBD samples	
			UC	CD
1	Cation barrier[35]	↑[120]	↑[86,119]	↑[86]
2	Cation pore and paracellular water channel[37,43,61]	–	↑[25,86,118]	↑[26,86]
3	Cation barrier[15]	–	↓[25]	↓[25]
4	Cation barrier[36]	–	↓[25,86]	–
5	Cation barrier[37]	–	–	↓[26]
7	Anion barrier and pore[38,39,40]	–	↓[86]	–
8	Cation barrier[54]	–	–	↓[26]
12	Barrier		–	↓[74]
18	Barrier[15]	↑ TNBS[127]	↑[128]	–

NOTE: UC, ulcerative colitis; CD, Crohn's disease; TNBS, trinitrobenzenesulfonic acid.

Table 2

Cytokine effects on claudin expression in IEC lines.

Cytokine	Claudin expression	Cells	References
TNF-α	↑ Claudin-1	IEC-18	119
TNF-α	↑ Claudin-2	HT-29/B6	100
TNF-α	↓ Claudin-2	T84	134
TNF-α	No change in claudin-2	T84	129
INFγ + TNF-α	↓ Claudin-2 ↓ Claudin-3	T84	25
IL-1β	↑ Claudin-1 ↓ Occludin	Caco-2	132
IL-4	↑ Claudin-2	T84	134
IL-6	↑ Claudin-2	Caco-2	131
IL-6	↑ Claudin-2	Caco-2	133
IL-13	↑ Claudin-2	T84	129
IL-13	↑ Claudin-2 ↑ Claudin-4	T84	25
IL-17	↑ Claudin-1 mRNA ↑ Claudin-2 mRNA	T84	130

Cell lines: HT-29/B6 and Caco-2, human colorectal carcinoma; IEC-18, non transformed small intestine; T84, colorectal carcinoma form lung metastasis.