



Review article

Autophagy and tight junction proteins in the intestine and intestinal diseases



Chien-An A. Hu^{a,b,*}, Yongqing Hou^{b,*}, Dan Yi^b, Yinsheng Qiu^b, Guoyao Wu^{b,c}, Xiangfeng Kong^d, Yulong Yin^{d,*}

^a Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, Albuquerque 87131, USA

^b Hubei Collaborative Innovation Center for Animal Nutrition and Feed Safety, Wuhan Polytechnic University, Wuhan 430023, China

^c Department of Animal Science, Texas A&M University, College Station 77843, USA

^d Institute of Subtropical Agriculture, Chinese Academy of Engineering, Changsha 410125, China

ARTICLE INFO

Article history:

Received 17 August 2015

Accepted 25 August 2015

Available online 1 September 2015

Keywords:

Autophagy

Claudins

IBD

Intestinal diseases

Proinflammatory cytokines

Tight junction barrier

ABSTRACT

The intestinal epithelium (IE) forms an indispensable barrier and interface between the intestinal interstitium and the luminal environment. The IE regulates water, ion and nutrient transport while providing a barrier against toxins, pathogens (bacteria, fungi and virus) and antigens. The apical intercellular tight junctions (TJ) are responsible for the paracellular barrier function and regulate trans-epithelial flux of ions and solutes between adjacent cells. Increased intestinal permeability caused by defects in the IE TJ barrier is considered an important pathogenic factor for the development of intestinal inflammation, diarrhea and malnutrition in humans and animals. In fact, defects in the IE TJ barrier allow increased antigenic penetration, resulting in an amplified inflammatory response in inflammatory bowel disease (IBD), necrotizing enterocolitis and ischemia-reperfusion injury. Conversely, the beneficial enhancement of the intestinal TJ barrier has been shown to resolve intestinal inflammation and apoptosis in both animal models of IBD and human IBD. Autophagy (self-eating mechanism) is an intracellular lysosome-dependent degradation and recycling pathway essential for cell survival and homeostasis. Dysregulated autophagy has been shown to be directly associated with many pathological processes, including IBD. Importantly, the crosstalk between IE TJ and autophagy has been revealed recently. We showed that autophagy enhanced IE TJ barrier function by increasing transepithelial resistance and reducing the paracellular permeability of small solutes and ions, which is, in part, by targeting claudin-2, a cation-selective, pore-forming, transmembrane TJ protein, for lysosome (autophagy)-mediated degradation. Interestingly, previous studies have shown that the inflamed intestinal mucosa in patients with active IBD has increased claudin-2 expression. In addition, inflammatory cytokines (for example, tumor necrosis factor- α , interleukin-6, interleukin-13, and interleukin-17) whose levels are increased in IBD patients cause an increase in claudin-2 expression and a claudin-2-dependent increase in TJ permeability. Thus, the role of claudin-2 in intestinal pathological processes has been attributed, in part, to the increase of intestinal TJ permeability. Claudin-2 represents a new therapeutic target in treating IBD, diarrhea and malnutrition in animals and humans.

© 2015 Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. The gastrointestinal epithelium in digestion, absorption and defense

In humans and animals, the ingested food and fluid are first processed by the enzymes in the saliva, then the acid in the stomach, and then the enzymes in the lumen and in the intestinal epithelium (IE). The IE is an indispensable barrier and interface between the gastrointestinal interstitium and the luminal environment that regulates water, ion and nutrient transport and absorption while providing a barricade against toxins, pathogens (bacteria, fungi and viruses) and antigens. The selective absorption

* Corresponding author.

E-mail addresses: AHu@salud.unm.edu (C.-A. Hu), Houyq@aliyun.com (Y. Hou), YinYulong@isa.ac.cn (Y. Yin).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

of digested nutrients, ions and water is achieved by transcellular transporters, co-transporters and channels of the microvillus and the apical membrane (Nigot et al., 2015; Shen et al., 2011; Suzuki, 2013; Turner, 2009). Nevertheless, the paracellular permeability of two adjacent enterocytes is achieved by the intercellular junctions that are sealed by, at least, four different types of protein complexes, tight junctions (TJ), adherens junctions, desmosomes and gap junctions. The TJ multiple protein complexes are located at the apical ends of the two lateral membranes of the IE. The TJ barrier (TJB) consists of transmembrane and intracellular scaffold proteins—at least four integral transmembrane proteins, occludin, claudins, MarvelD3 or junctional adhesion molecule (JAM), and tricellulin, have been identified (Turner, 2009). The claudin family has 27 members. The extracellular loops of claudins form a selective barrier in the paracellular pathways with adjacent cells, and the intracellular domains interact with scaffold proteins such as zonula occludens (ZO) proteins and cingulin, which in turn anchor the transmembrane proteins to the actin cytoskeleton. Myosin light chain kinase (MLCK) is associated with the perijunctional actomyosin rings and regulates paracellular permeability through myosin contractility (Amasheh et al., 2002; Furuse et al., 1998, 2001; Gunzel and Yu, 2013; Krug et al., 2014; Rosenthal et al., 2010).

2. The apical intercellular tight junctions, claudins and their associated diseases

The apical intercellular TJ are responsible for the paracellular barrier function and regulate trans-epithelial flux of ions and solutes between adjacent cells. Increased intestinal permeability caused by defects in the IE TJB is considered an important pathogenic factor for the development of intestinal inflammation, diarrhea and malnutrition in humans and animals. In fact, defects in the IE, TJB allow for increased antigenic penetration, resulting in an amplified inflammatory response in inflammatory bowel disease (IBD), necrotizing enterocolitis and ischemia-reperfusion injury. The IE and TJ are integrators of mucosal homeostasis. Tight junction barrier defects allow bacterial products/enterotoxins and dietary and luminal antigens to cross the IE and enter the lamina propria. If the foreign substances are taken up by antigen-presenting cells (APC), APC and Th1 cells can release tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ), which signal to IE to increase the TJ leak flux pathway. Leaky TJB allows for the translocation of bacterial products and dietary antigens from the lumen into the lamina propria, which amplifies the cycle of inflammation, ultimately leading to intestinal disease. Alternatively, interleukin-13 (IL-13) released by Th2 cells increases flux across small cation-selective pores, potentially contributing to ongoing disease. Conversely, the beneficial enhancement of the IE TJB activity, for example, treatment with anti-TNF- α antibody, has been shown to resolve intestinal inflammation and apoptosis in both animal models of IBD and human IBD (Heller et al., 2005; Oshima et al., 2008; Prasad et al., 2005; Schmitz et al., 1999; Weber et al., 2008).

Claudins are transmembrane proteins with a molecular weight of 20 to 27 kDa. Since 1998, twenty seven human genes coding for claudin proteins have been found (Krug et al., 2014; Turner, 2009). Claudins, together with other protein components (for example, occludins, tricellulin and MarvelD3) form TJ in the epithelium and the endothelium. The general functions of claudins are: (a) formation of barrier or channel/pore, (b) regulation of cellular polarity, signaling, proliferation, differentiation, receptor function, and motility, and (c) boundary establishment for limiting the intermixing of lateral and apical membrane proteins. Claudins in TJB prevent unregulated passage of solutes and water as well as

penetration of luminal toxins and antigens. At the same time, claudins in TJB control paracellular permeation, both absorptive or secretory transport, by: (a) water selectivity, (b) cation selectivity, and (c) anion selectivity (Rosenthal et al., 2010). Alterations in abundance or molecular structure of claudins can generally result in three typical effects: (a) decreased absorption, (b) increased secretion of water and small solutes causing leak flux diarrhea, and (c) increased absorption of macromolecules which may induce inflammatory response and result in intestinal inflammation and symptoms like weight loss, abdominal pain or diarrhea.

Claudins show differential expression patterns throughout the intestine. In intestinal diseases, claudins are involved in alterations of expression as well as localization and distribution along the lateral membrane and the intercellular space. These alterations may lead to severe disturbances in the regulation of water, ion and solute transport as well as macromolecule uptake in affected areas, resulting in the specific clinical phenotype, such as IBD and Celiac disease. Table 1 lists the changes of claudins in major intestinal diseases (Lu et al., 2013; Milatz et al., 2010; Suzuki et al., 2011; Thuijls et al., 2010; Zeissig et al., 2007).

2.1. Inflammatory bowel disease

Inflammatory bowel disease comprises two major entities, Crohn's disease (CD) and ulcerative colitis (UC). In CD, the whole intestinal tract from the oral cavity to the colon can be infested. In contrast, UC is limited to the colon spreading from distal to proximal and always affects the rectum. In both diseases, extra-intestinal manifestations can occur, affecting joints, liver and skin. Patients mostly suffer from episodic inflammation with frequent, often bloody diarrhea and abdominal pain. Both diseases particularly affect younger persons in their 30's and 40's, but older persons can also be affected, known as late onset IBD (Heller et al., 2005; Weber et al., 2008).

2.2. Crohn's disease

Crohn's disease is characterized by a dysregulated IE TJB function. Epithelial resistance was reduced by about 40% in colonic biopsies of CD patients with mild to moderate inflammation. Crohn's disease is presented with reduced and discontinuous TJ strands and altered claudin expression and localization (Table 1). As reported, claudin-2 expression was significantly increased and localized to crypt bases of the colon and the duodenum of active human CD patients (Goswami et al., 2014; Zeissig et al., 2007). It is thought that CD exhibits a predominant Th1 immune response, in which proinflammatory cytokines TNF- α and IFN- γ are dominant. Nevertheless, studies have shown that TNF- α and IFN- γ induce claudin-2 expression in HT-29/B6 and Caco-2 cells (Zeissig et al., 2007), where transepithelial electrical resistance (TER) decreases and cation permeability increases. In contrast, claudin-3 expression and localization were reduced in the inflamed colon of CD

Table 1
Expression of various claudins in human intestinal diseases.

| Inflammatory bowel disease | Upregulated | Downregulated | Reference |
|----------------------------|-------------|----------------------|---|
| Crohn's disease | Claudin-2 | Claudin-3, -5 and -8 | Goswami et al., 2014; Zeissig et al., 2014 |
| Ulcerative colitis | Claudin-2 | Claudin-3, -4 and -7 | Heller et al. 2005; Prasad et al., 2005; Weber et al., 2008 |
| Irritable bowel syndrome | Claudin-2 | Claudin-1 and -4 | Martínez et al., 2013 |
| Celiac disease | Claudin-2 | Claudin-3, -5 and -7 | Schumann et al., 2012; Szakál et al., 2010 |

patients. In addition, claudin-3 showed a diffuse cytoplasmic staining instead of an intense and predominantly lateral staining in CD patients (Goswami et al., 2014; Zeissig et al., 2007). Thus, alterations in TJ structure with barrier impairment in CD patients result from up-regulation of the channel-forming protein claudin-2 and down-regulation and redistribution of the sealing proteins claudin-3, -5 and -8.

2.3. Ulcerative colitis

In active UC patients, the epithelial resistance is reduced by about 80% (Schmitz et al., 1999). Concomitantly the depth of the TJ meshwork and the number of TJ strands was significantly reduced, which indicated a marked alteration in TJ composition, resulting in a severely impaired TJB function with a leak-flux mechanism. An uncontrolled exchange and loss of water and solutes in combination with increased antigen uptake triggers the chronic inflammatory cascade. Interestingly, claudin-2 has been shown to be upregulated by IL-13, TNF- α , and IL-6 in active IBD patients. These three cytokines are present in UC patients as part of a predominant Th2 immune response. Interleukin-13 and TNF- α , in addition to stimulating claudin-2 expression, also increased IE apoptotic rate (Schmitz et al., 1999; Oshima et al., 2008). Interleukin-6 has been shown to stimulate claudin-2 expression through the MEK/ERK and PI3K pathways in Caco-2 cells (16). In contrast, claudin-3, a tightening protein, has been shown to be significantly downregulated in biopsies from active UC patients (Heller et al., 2005; Prasad et al., 2005; Thuijls et al., 2010; Weber et al., 2008). Interestingly, claudin-2 also has been found to be upregulated in patients with Celiac disease (Schumann et al., 2012; Szakál et al., 2010) and irritable bowel syndrome (Martínez et al., 2013) (Table 1).

3. Autophagy

Macroautophagy (self-eating; from here on autophagy is used) is an intracellular lysosome-dependent degradation and recycling pathway essential for cell survival and homeostasis. Autophagy rids the aged and damaged cells and helps in the elimination of pathogens, and thus is important during normal development as well as in response to environmental stimuli. Autophagy possesses several cellular characteristics, for example, the formation of distinct interactomes and structures (e.g., isolation membrane, autophagic vesicles). It has three major phases: initiation, elongation and completion. Initiation involves the formation of a double membrane structure (isolation membrane to autophagosome), which captures a portion of the cytoplasm, organelles, and intracellular pathogens, if any, and then fuses with endosomes and/or lysosomes (becoming the amphisome and the autolysosome, respectively) to degrade the contents of the vesicle (Hu et al., 2012; Levine et al., 2011; Mizushima and Komatsu, 2011). The formation of this double membrane structure is a complex process involving many AuTophagy-related proteins (Atg). Currently, over 30 Atg have been identified, including Beclin 1 (Atg 6). LC3 (Atg8) is required for the formation of autophagosomal membranes. Beclin 1 complex and then LC3 are recruited to the isolation membrane, which will ultimately develop into the autophagosome where cellular targets are sequestered. The autophagosome will then fuse with endosomes/lysosomes to create the autolysosome where cellular targets are degraded. Under nutrient-deprived conditions, autophagy can be induced at the transcriptional and post-translational level. With regard to post-translational protein modification, LC3, for example, exists in both a cytoplasmic and a (autophagic) membrane-associated form. The process of incorporating LC3 to the membrane is accomplished through LC3

cleavage, lipidation and translocation. The LC3 designation is modified once cleavage has occurred as well as when the protein is localized to the membrane. Under this nomenclature, LC3-I refers to a cytosolic polypeptide formed from LC3 cleavage and LC3-II refers to a membrane-bound LC3. Atg4, a specific cysteine protease belonging to the caspase family, initiates LC3 processing by post-translationally cleaving LC3's C-terminal amino acid (arginine). This cleavage generates LC3-I. The newly exposed C-terminal glycine (Gly 116) is bound by phosphatidylethanolamine (PE), a lipid constituent of plasma membranes, followed by lipidation to form LC3-II. LC3-II associates with both the inner and outer membranes of the isolation membrane. Transient conjugation of LC3 to the autophagosomal membrane through a ubiquitin like system is essential for autophagy. The conversion of LC3-I to LC3-II has been used as an indicator of autophagic state in *in vitro* model systems. In addition, autophagy uses two essential conjugation systems/complexes, Atg7 (E1-like), Atg3 and Atg10 (E2-like), and Atg5-Atg12-Atg16L (E3-like), for the successful progression of autophagosome elongation and completion, similar to the ubiquitin targeting system (Hu et al., 2012; Levine et al., 2011; Mizushima and Komatsu, 2011).

4. Autophagy regulates TJB function and paracellular permeability, in part, through normalizing the homeostatic level of claudin-2

Our recent investigation has revealed the crosstalk between autophagy and IE TJB. Using cultured IE Caco-2 (human) and MDCKII (dog) cell lines in combination with various autophagy inducers (starvation, mTOR inhibitors), inhibitors (si-Claudin-2), overexpression system, and assays (cell count, TER measurement, solute transport, immunoblot, immune-fluorescence microscopic analyses), we showed that autophagy enhanced IE TJB function by increasing TER and reducing the paracellular permeability of small solutes and ions, which is, in part, by targeting claudin-2 for lysosome (autophagy)-mediated degradation. Importantly, claudin-2 has been shown to be a cation-selective, pore-forming, transmembrane TJ protein. Our results strongly suggest that claudin-2 plays a major role in inflammatory intestinal diseases and its level can be fine tuned by healthy autophagic mechanism (Fig. 1).

5. Conclusive remarks and future perspectives

Results of recent studies have shown that the inflamed intestinal mucosa in patients with active IBD, both CD and UC, has increased claudin-2 expression (Table 1). In addition,

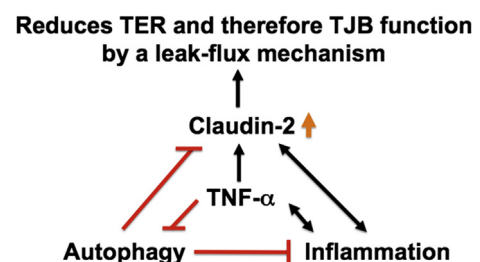


Fig. 1. Crosstalk between autophagy, claudin-2, inflammation, and TNF- α . Claudin-2 plays a major role in inflammatory intestinal diseases and its level can be fine tuned by healthy autophagic mechanism. However, inflammation and TNF- α both can block autophagy and simultaneously induce claudin-2 overexpression which cause reduction of TER and TJB function by a leak-flux mechanism. TER = transepithelial electrical resistance; TJB = tight junction barrier; TNF- α = tumor necrosis factor- α .

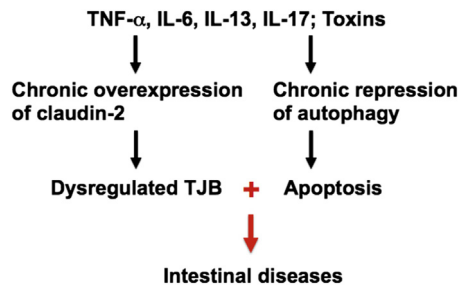


Fig. 2. Inflammatory Cytokines, such as TNF- α , IL-6, IL-13, IL-17, induce chronic inhibition of autophagy and induction of claudin-2 expression, leading to apoptosis and dysregulated TJ barrier function and subsequent intestinal diseases. Autophagy fine tunes claudin-2 homeostasis. TNF- α = tumor necrosis factor- α ; IL-6 = interleukin-6; IL-13 = interleukin-13; IL-17 = interleukin-17; TJB = tight junction barrier.

inflammatory cytokines (for example, TNF- α , IL-6, IL-13, and IL-17) whose levels are increased in IBD patients cause an increase in claudin-2 expression and a claudin-2-dependent increase in TJ permeability. Thus, the role of claudin-2 in intestinal pathological processes has been attributed, in part, to the increase of intestinal TJ permeability (Fig. 1 and Fig. 2). Consistence with these observations, claudin-2 knockout MDCK cell line and mouse model also showed increased TER and paracellular Na⁺ permeability (Tamura et al., 2011; Wada et al., 2013). Interestingly, recent genome-wide association studies have identified several genes implicated in autophagy (ATG16L1, IRGM, ULK1), intracellular bacterial sensing (NOD2), endoplasmic reticulum (ER) stress (XBP1 and ORMDL3), and claudin-2 to be associated with CD. Thus, claudin-2 represents a new therapeutic target in treating intestinal diseases, diarrhea and malnutrition in animals and humans, and the molecular basis of autophagy-induced claudin-2 degradation is an important subject for our current study. Hypothetically, we have proposed a model that claudin-2 degradation is through an active autophagy (Fig. 3). This new knowledge has important implications for developing nutritional and pharmacological means to improve intestinal protein homeostasis and health in humans and animals.

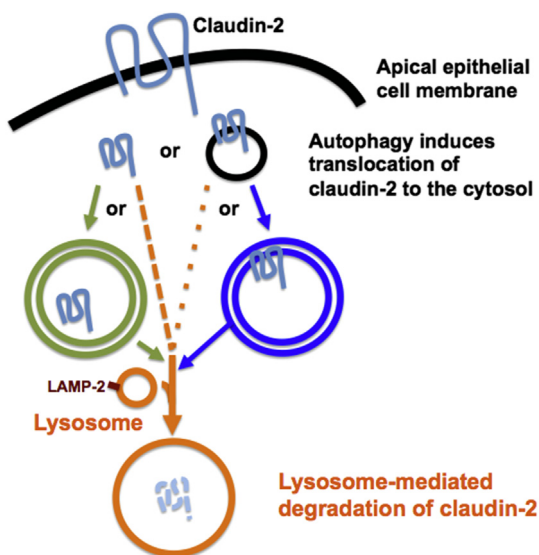


Fig. 3. Hypothetical model of how claudin-2 is degraded through autophagy- and/or lysosome-mediated degradation. As claudin-2 is a transmembrane protein, its translocation to lysosomes by way of the cytosol may involve a portion of the membrane or not. Cytosolic claudin-2 may be enwrapped in an autophagosome (green double membrane structure) or directly engulfed by a lysosome. The molecular mechanism of claudin-2 degradation is currently under investigation.

Acknowledgments

We are grateful to past and present members of our research groups, our collaborators, and many others in the field. We apologize to colleagues whose outstanding work on tight junction functions and physiology in intestinal diseases was not cited due to length and journal restrictions. Our work is supported, in part, by the Evergreen Scholarship of the Wuhan Polytechnic University (to CAAH) and Texas A&M AgriLife Research (H-8200 to GW), and National Natural Science Foundation of China (No. 31372319 and 31402084) (to YH).

References

- Amasheh S, Meiri N, Gitter AH, Schoneberg T, Mankertz J, Schulzke JD, et al. Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J Cell Sci* 2002;115:4969–76.
- Furuse M, Fujita K, Hiiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occluding. *J Cell Biol* 1998;141:1539–50.
- Furuse M, Furuse K, Sasaki H, Tsukita S. Conversion of zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin–Darby canine kidney I cells. *J Cell Biol* 2001;153:263–72.
- Goswami P, Das P, Verma AK, Prakash S, Das TK, Nag TC, et al. Are alterations of tight junctions at molecular and ultrastructural level different in duodenal biopsies of patients with celiac disease and Crohn's disease? *Virchows Arch* 2014;465:521–30.
- Gunzel D, Yu AS. Claudins and the modulation of tight junction permeability. *Physiol Rev* 2013;93:525–69.
- Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology* 2005;129:550–64.
- Hu CA, Klopfer EI, Ray PE. Human apolipoprotein L1 (ApoL1) in cancer and chronic kidney disease. *FEBS Lett* 2012;586:947–55.
- Krug SM, Schulzke JD, Fromm M. Tight junction, selective permeability, and related diseases. *Semin Cell Dev Biol* 2014;36:166–76.
- Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature* 2011;469:323–35.
- Lu Z, Ding L, Lu Q, Chen YH. Claudins in intestines: distribution and functional significance in health and diseases. *Tissue Barriers* 2013;1:e24978.
- Martínez C, Lobo B, Pigrau M, Ramos L, González-Castro AM, Alonso C, et al. Diarrhea-predominant irritable bowel syndrome: an organic disorder with structural abnormalities in the jejunal epithelial barrier. *Gut* 2013;62:1160–8.
- Milatz S, Krug SM, Rosenthal R, Gunzel D, Müller D, Schulzke JD, et al. Claudin-3 acts as a sealing component of the tight junction for ions of either charge and uncharged solutes. *Biochim Biophys Acta* 2010;1798:2048–57.
- Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011;147:728–41.
- Nigot P, Hu CAA, Ma T. Autophagy enhancement of intestinal epithelial tight junction barrier function by targeting claudin-2 degradation. *J Biol Chem* 2015;290:7234–46.
- Oshima T, Miwa H, Joh T. Changes in the expression of claudins in active ulcerative colitis. *J Gastroenterol Hepatol* 2008;23(Suppl. 2):S146–50.
- Prasad S, Mingrino R, Kaukinen K, Hayes KL, Powell RM, MacDonald TT, et al. Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Lab Invest* 2005;85:1139–62.
- Rosenthal R, Milatz S, Krug SM, Oelrich B, Schulzke JD, Amasheh S, et al. Claudin-2, a component of the tight junction, forms a paracellular water channel. *J Cell Sci* 2010;123:1913–21.
- Schmitz H, Barmeyer C, Fromm M, Runkel N, Foss HD, Bentzel CJ, et al. Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. *Gastroenterology* 1999;116:301–9.
- Schumann M, Gunzel D, Buerger N, Richter JF, Troeger H, May C, et al. Cell polarity-determining proteins Par-3 and PP-1 are involved in epithelial tight junction defects in coeliac disease. *Gut* 2012;61:220–8.
- Shen L, Weber CR, Raleigh DR, Yu D, Turner JR. Tight junction pore and leak pathways: a dynamic duo. *Annu Rev Physiol* 2011;73:283–309.
- Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci* 2013;70:631–59.
- Szákál DN, Györfy H, Arató A, Cseh A, Molnár K, Papp M, et al. Mucosal expression of claudins 2, 3 and 4 in proximal and distal part of duodenum in children with coeliac disease. *Virchows Arch* 2010;456:245–50.
- Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. *J Biol Chem* 2011;286:31263–71.
- Tamura A, Hayashi H, Imasato M, Yamazaki Y, Hagiwara A, Wada M, et al. Loss of claudin-15, but not claudin-2, causes Na⁺ deficiency and glucose malabsorption in mouse small intestine. *Gastroenterology* 2011;140:913–23.

- Thuijls G, Derikx JP, de Haan JJ, Grootjans J, de Bruijne A, Masclee AA, et al. Urine-based detection of intestinal tight junction loss. *J Clin Gastroenterol* 2010;44:e14–9.
- Turner JR. Intestinal mucosa barrier function in health and disease. *Nat Rev Immunol* 2009;9:799–809.
- Wada M, Tamura A, Takahashi N, Tsukita S. Loss of claudins 2 and 15 from mice causes defects in paracellular Na⁺ flow and nutrient transport in gut and leads to death from malnutrition. *Gastroenterology* 2013;144:369–80.
- Weber CR, Nalle SC, Tretiakova M, Rubin DT, Turner JR. Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. *Lab Invest* 2008;88:1110–20.
- Zeissig S, Burgel N, Gunzel D, Richter J, Mankertz J, Wahnschaffe U, et al. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut* 2007;56:61–72.