World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v22.i11.3117

World J Gastroenterol 2016 March 21; 22(11): 3117-3126
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer

Jonathan Landy, Emma Ronde, Nick English, Sue K Clark, Ailsa L Hart, Stella C Knight, Paul J Ciclitira, Hafid Omar Al-Hassi

Jonathan Landy, Ailsa L Hart, IBD Unit, St Mark's Hospital, Harrow, London HA1 3UJ, United Kingdom

Jonathan Landy, Emma Ronde, Nick English, Ailsa L Hart, Stella C Knight, Hafid Omar Al-Hassi, Antigen Presentation Research Group, Department of Immunology, Imperial College, London HA1 3UJ, United Kingdom

Sue K Clark, Department of Biosurgery and Surgical Technology, Imperial College, London HA1 3UJ, United Kingdom

Paul J Ciclitira, Department of Gastroenterology, The Rayne Institute, St Thomas' Hospital, London HA1 3UJ, United Kingdom

Author contributions: Landy J and Al-Hassi HO contributed equally to the conception of the article, literature search and interpretation, writing the article and making critical revisions related to important intellectual content of the manuscript, and final approval of the version of the article to be published; Ronde E contributed to the literature search, drafting of the article and final approval of the version of the article to be published; English N was responsible for the electron micrographs presented in the figures; Clark SK, Knight SC, Hart AL and Ciclitira PJ made critical revisions related to important intellectual content of the manuscript, and the final approval of the version of the article to be published.

Supported by The Association for International Cancer Research (AICR, to Dr. Al-Hassi HO), Scotland; Funded by the AICR, grant No. 120234; a BBSRC Strategic Research Grant (to English N and Knight SC; WMNIP33458); the St Mark's Hospital Foundation, United Kingdom.

Conflict-of-interest statement: None of the authors have any conflicts of interest or financial disclosures; and Dr. Hart has spoken for and is on the advisory board for MSD, Shire and Abbott.

Correspondence to: Paul J Ciclitira, Professor, Department of Gastroenterology, The Rayne Institute, St Thomas' Hospital, 4th Floor Lambeth Wing, London SE1 7EH, United Kingdom. paul.ciclitira@kcl.ac.uk
Telephone: +44-20-76202597
Fax: +44-20-72610667

Received: June 18, 2015
Peer-review started: June 21, 2015
First decision: August 26, 2015
Revised: September 19, 2015
Accepted: December 19, 2015
Article in press: December 19, 2015
Published online: March 21, 2016

Abstract

Inflammatory bowel diseases are characterised by inflammation that compromises the integrity of the epithelial barrier. The intestinal epithelium is not only a static barrier but has evolved complex mechanisms to control and regulate bacterial interactions with the mucosal surface. Apical tight junction proteins are critical in the maintenance of epithelial barrier function and control of paracellular permeability. The characterisation of alterations in tight junction proteins as key players in epithelial barrier function in inflammatory bowel diseases is rapidly enhancing our understanding of critical mechanisms in disease pathogenesis as well as novel therapeutic opportunities. Here we give an overview of recent literature focusing on the role of tight junction proteins, in particular claudins, in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer.

Key words: Claudin; Tight junction; Ulcerative colitis; Pouchitis; Crohn's disease

Core tip: Epithelial barrier function is compromised in inflammatory bowel diseases (IBD). Apical tight junction proteins, in particular claudins, are key players in epithelial barrier function. However, there is little consensus regarding the expression of most claudin isoforms in these conditions or whether these findings are primary or secondary to disease pathogenesis. Knowledge of tight junction protein expression and function in IBD and IBD associated colorectal cancer will enhance our understanding of critical mechanisms in disease pathogenesis as well as novel therapeutic opportunities.

Landy J, Ronde E, English N, Clark SK, Hart AL, Knight SC, Ciclitira PJ, Al-Hassi HO. Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer. *World J Gastroenterol* 2016; 22(11): 3117-3126 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i11/3117.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i11.3117

INTRODUCTION

A number of routes exist for passage across the epithelial barrier between the internal and external environment. The intercellular spaces between adjacent cells, linked together by junctional complexes, are critical in regulating the mucosal barrier. Ions and solutes pass transcellulary utilising channels, carriers and transporting ATPases[1]. The paracellular pathway also regulates permeability to water, ions and low molecular weight molecules (< 600 kDa). The differences in paracellular permeability between different epithelia such as the proximal or distal nephron, has led to the concept of "leaky" or "tight" epithelia. In disease states, epithelia may become more "tight" or more "leaky" such as is seen in inflammatory bowel diseases[1].

The tight junctions (TJs) form the apical most unit, defining the boundary between the apical and basolateral membranes and are predominantly the rate-limiting factor in paracellular passage[2]. The tight junction is built up by both transmembrane proteins such as occludin, tricellulin, different claudins and junctional adhesion molecules (JAMs), as well as peripheral membrane proteins such as zona occludens (ZO)-1,-2,-3 and cingulin. They are linked to the cytoskeleton of the cell by F-actin and myosin Ⅱ[3,4]. These ZO proteins have three PDZ domains that mediate binding to other transmembrane tight junction proteins such as claudins in a dynamic energy dependent manner[5]. They are also the direct targets and effectors of different signalling pathways (such as the myosin light chain kinase) thereby altering the assembly, maintenance, and barrier function of the TJ complex[6].

The expression of different TJs in the gut varies according to localization (*e.g.*, villus *vs* crypt, small bowel *vs* colon), cell membrane localization (*e.g.*, apical, lateral or basolateral) and the gut's functional properties at the site[4,7,8]. For example, claudin 2 is expressed at the apical pole throughout the crypt-villus axis in the jejunum whilst in the colon expression is restricted to the crypts, whereas claudin 4 expression is throughout the crypt-villus axis in the small and large intestine. The segmental distribution of claudin expression may relate to cell differentiation, carbohydrate metabolism and transcription factors such as HNF1α, Cdx2 and GATA-4[9].

In health, the apical TJs construct a dynamic intestinal barrier that regulates the paracellular uptake of water, nutrients and electrolytes[3,5]. TJs may be size and/or charge selective and prevent contact between the proteins of the two cell poles: the basolateral and apical cell membranes[10]. While many tight junction proteins have properties of increased barrier formation, others form size and/or charge selective channels or pores[1]. Adherens junctions and desmosomes are mostly involved in communication between neighbouring epithelial cells[4,10,11]. TJ dysfunction can lead to the disruption of the intestinal barrier integrity. Changes in pH, osmotic load or cytoskeleton function all affect the barrier function of TJs[12].

There are 27 different claudin isoforms that modulate the paracellular movement of ions based on charge and size[13]. Claudin 1 and claudin 2 are able to initiate the formation of TJ strands on fibroblasts lacking TJs, suggesting that they are the major components of TJ strands[14]. Claudin 2 controls the movement of monovalent cations such as Na^+ to the interstitium and reduces the paracellular trans-epithelial resistance as well as enhances trans-epithelial water flux[15,16] in contrast to other claudins (like 1, 3, 4, 5, 8) that tighten the epithelium[17-23]. Claudin 2 also directly decreases the barrier function of Claudin 1 and Claudin 4 strands[13]. Therefore the ratio of different claudins in the TJ determines its functional property as either leaky or tight. This review will focus on the role of key TJPs (Table 1) in the pathogenesis of IBD and IBD associated colorectal cancer (CRC).

EPITHELIAL BARRIER FUNCTION IN INFLAMMATORY BOWEL DISEASES

Inflammatory bowel diseases (IBD) share a multifactorial aetiology of genetic susceptibility, environmental factors and immune dysregulation[24]. These diseases are characterized by intestinal inflammation that compromises the integrity of the epithelial barrier leading to increased permeability

Table 1 Tight junctions of interest in inflammatory bowel diseases

TJ protein	Function	Expression in CD	Expression in UC	Expression in pouchitis
Claudin 1	Tightens the epithelium[17,23] able to initiate formation of TJ strands[14]	Active: ↑[46,65] ↔[50] Inactive: ↔[65]	Active: ↑[65], ↔[45] Inactive: ↔[65]	↓[40]
Claudin 2	Important pore-forming TJ protein[15,16,23], able to initiate formation of TJ strands[14], decreases barrier function of CLDN1 and CLDN4[13]	↑[44,50,65,101]	↑↑[44,50,65,101]	↑[40]
Claudin 4	Tightens the epithelium, decreases paracellular conductance through decrease in sodium permeability[19]	↓[44] Active inflammation: ↑[65] ↔[50]	Active: ↓[44,45] Active Inflammation: ↑[65]	↔[40]
Claudin 5	Tightens the epithelium[20]	↓[50]		↔[40]
Claudin 8	Tightens the epithelium[21]	↓[50]		
Claudin 12	Tightens the epithelium	↑[48]		
Claudin 18	Uncertain function		↑[47]	
Occludin	Binds ZO-1, regulates paracellular permeability, function in cellular adhesion[102]	↓[46,50,52] ↔[65]	↓[52] ↔[65]	↔[40]
ZO-1	Mediates protein-protein interactions, link to actin cytoskeleton: "anchoring" protein[4,103]	↓[68]	↔[64] (Mees *et al*: "in patients with a history of UC")	↓[54] (chronic pouchitis)

TJ: Tight junction; ZO-1: Zona occludens-1; CD: Crohn's disease; UC: Ulcerative colitis.

and infiltration of pathogens[25]. Both Crohn's disease (CD) and ulcerative colitis (UC) share common features such as epithelial breaks, a reduction in tight junction strands, and glandular atrophy[11,26,27]. Patients with clinically active CD have increased intestinal permeability[28-30]. Barrier dysfunction is likely to be caused by epithelial damage including apoptosis, erosion and ulceration that are characteristic of gut inflammation. Inflammatory cytokines associated with gut inflammation alter epithelial permeability through their effects on the junctional complexes[31-33].

However, impaired barrier function is also evident in quiescent IBD and even in first degree relatives of patients with CD[34,35]. Genetic studies have identified new UC susceptibility loci pertaining to defects of the epithelial barrier[36,37]. Barrier properties of ileoanal pouch mucosa in both pouchitis and in ileoanal pouches where backwash ileitis was present prior to restorative proctocolectomy for UC are reduced[38-40] and increased bacterial translocation has been reported in pouches functioning for longer than 12 mo[41]. Dysregulation of the epithelial barrier with changes in paracellular permeability due to altered cell to cell junctions is likely to be significantly more selective and may be a critical primary factor in the pathogenesis of IBD.

ULCERATIVE COLITIS

Gitter *et al*[42] identified that in the sigmoid colon of patients with early UC where the epithelium looks intact, there are in fact already leaks from apoptotic foci. Furthermore, the higher the degree of inflammation observed, the higher the conductance of the epithelium measured[42]. A study using epithelial resistance as a measure of barrier function in samples from UC patients with inflamed sigmoid colon[43] demonstrated an 80% reduction in epithelial resistance and a decrease in epithelial (not crypt) TJ depth in inflamed samples.

Several studies have focused on expression of claudins in UC patients[43-45]. demonstrating higher expression of claudin 2 in colonic samples from patients with UC. Additionally, the increases in "pore-forming" claudin 2 correlate with disease severity on both protein and transcriptional levels. Reductions in other "tightening" tight junctions also occur concomitantly. Reduced staining intensity for claudin 3, 4 and 7 have been shown both on the surface epithelium as well as mislocation of claudin 4 extra-junctionally in UC patients[44,45].

In contrast, Poritz *et al*[46] found an increase in claudin 1:occludin ratios in colonic samples from UC patients compared with healthy controls and CD samples by Western blot analysis. This change in ratio was the result of both an increase in claudin 1 and a decrease in occludin. Disease severity, measured by the degree of inflammation, was directly proportional to the alterations seen in TJ structure in UC. In another study, claudin 1 was demonstrated to be upregulated in the colon of UC patients compared to healthy controls, but did not correlate to disease severity[47]. In sigmoid samples of active UC patients, a trend toward upregulation of claudin 12 (another "tightening" claudin) was observed[48]. Claudin 18 expression was found to be elevated in UC patients compared with controls, but did not correlate with severity of inflammation postulating a primary defect in barrier function[47].

CROHN'S DISEASE

In CD, intestinal permeability is considered a predictive

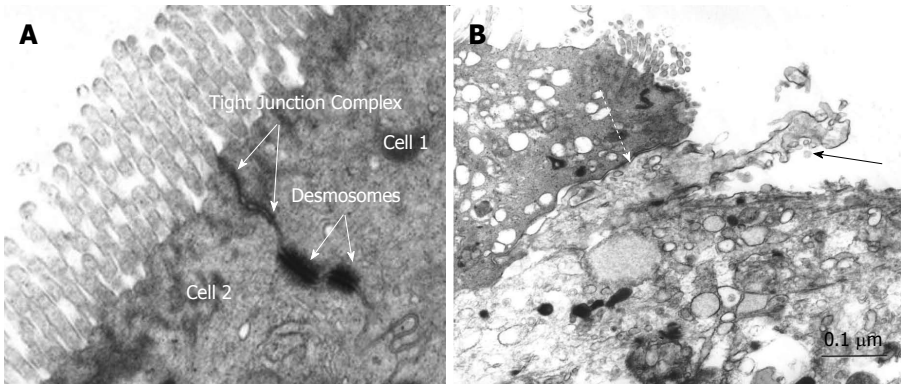

Figure 1 Electron microscopy of pouch epithelium tight junction complex in non-inflamed pouch (A) and electron micrograph demonstrating dendritic cell penetrating between two epithelial cells in pouchitis (B). Bar = 0.1 μm. Solid arrow: Dendritic cell; Dashed arrow: Tight junction complex between epithelial cell and dendritic cell.

factor for disease susceptibility and relapse[34,49]. Changes in tight junctions and epithelial apoptosis might dominate in causing barrier dysfunction[50]. Marin *et al*[51] studied 10 patients with CD. The epithelial cells of the terminal ileum from these patients demonstrated tight junction disorganisation which was present in minimally inflamed areas although more pronounced in cobblestone areas.

In samples from the sigmoid colon in active CD, the expression of claudins 5, 8 and 3 were decreased, whereas claudin 2 expression was moderately increased[50]. Kucharzik *et al*[52] demonstrated similar changes in tight junction expression in both UC and CD with global down-regulation of occludin. This finding was present both in active and quiescent UC whilst only in active CD. Das *et al*[53] showed claudin 2 to be strongly expressed in the ileum of approximately 50% of quiescent as well as active CD. Moreover, the distribution of claudin 2 expression was altered in colonic biopsies from CD patients and associated with disrupted tight junctions. Others have found no change in ileal expression of claudin 2 in CD, whilst in the sigmoid colon of CD patients, claudin 2 was found to be significantly down regulated compared to controls. However, claudin 12 was found to be increased in the ileum of CD patients[48].

POUCHITIS

Barrier properties of ileoanal pouch mucosa in pouchitis and in non-inflamed pouches are reduced[38] with increased bacterial translocation in long lasting pouches[41]. Merrett *et al*[39] showed an increase in pouch permeability in patients with pouchitis compared with those with a normally functioning pouch. Electron micrography of mucosal epithelium from non-inflamed UC pouch (Figure 1A) and pouchitis suggests membranes between cells are more loosely arranged with increased intercellular distance and perijunctional cytoskeleton condensation[54]. In addition, dendritic cells appear to penetrate the epithelial cell layer more frequently (personal communication) (Figure 1B). Analysis of claudin expression from pouch biopsies before ileal pouch anal anastomosis (IPAA), during pouchitis and at a time point over a year after ileostomy closure demonstrated an elevation in claudin 2 levels in acute pouchitis[40]. We also recently demonstrated altered expression of TJP in the ileal pouch of patients with UC. In particular increased expression of claudin 2 occurred early following ileostomy closure, prior to the development of histological inflammation. Claudin 2 was also elevated in UC patients without pouchitis compared with FAP patients and in acute, but not chronic pouchitis samples whilst epithelial expression of ZO-1 and claudin 1 were reduced in patients with chronic pouchitis. These findings suggest that increased claudin 2 expression may be an early event in the development of inflammation[54].

TIGHT JUNCTIONS AND IBD ASSOCIATED COLORECTAL CANCER

Passage of luminal antigens or metabolites through diminished epithelial barrier is known to promote chronic inflammation and CRC[55-57]. In general, claudins are associated with different types of neoplasms, including breast, prostate, ovarian, pancreatic, gastric and colorectal carcinoma[58,59]. They may also be involved in the progression to metastasis, and provide unfavourable signalling pathways between the extra- and intracellular milieu[60-63].

Mees *et al*[64] investigated the expression of both adherens, and TJ proteins in patients with CRC with a history of UC. Claudins 1, 3, 4 and β-catenin were upregulated in CRC tissue compared with normal controls or intraepithelial neoplasia, whilst the expression of claudin 2, ZO-1 and occludin did not vary significantly between samples. Other studies have demonstrated elevated claudin 1 and claudin 2 levels in IBD-associated carcinoma[59,65,66]. Kinugasa *et al*[66] demonstrated increased staining for claudin 1 in both high-grade dysplasia and UC-associated CRC when

compared with normal or UC colonic samples.

In the study of Weber *et al*[65], the expression of claudin 1, 2, 4 and occludin were evaluated in IBD patients, IBD-associated dysplasia, acute self-limited colitis (ASLC) and sporadic adenomas. During active inflammation, claudin 1 expression was upregulated in both UC and CD patients and localization of claudin 1 was not limited to the TJ, but was expressed on the lateral membrane. Claudin 2 expression was the richest in the TJ region and the apical cytoplasm. In an identical manner to claudin 1, the expression of claudin 2 correlated with the degree of inflammation in both UC and CD, but not in ASLC[65]. Claudin 1 and claudin 2 levels were also increased in IBD-associated dysplasia and sporadic adenomas in comparison to non-dysplastic IBD. However, occludin expression and localization in both active and inactive IBD and in adenomas or carcinomas was no different compared with controls. The changes in TJ expression in IBD associated dysplasia may represent the severity and longevity of histological inflammation, a known risk factor for neoplastic progression in IBD[67].

CHICKEN OR EGG?

It is not clear whether the changes observed in tight junctions in IBD are causal, leading to abnormal epithelial barrier integrity and the aberrant immune responses, or whether the inflammation itself causes the alterations in tight junction expression and distribution.

Inflammatory cytokines and tight junction regulation

Heller *et al*[31] demonstrated a significant upregulation of IL-13 in patients with UC compared to CD patients and non-inflammatory controls. Following the upregulation of IL-13, there was a decrease in trans-epithelial resistance and an increase in epithelial cell apoptosis and conductance. Furthermore, IL-13 caused an upregulation of the claudin 2 gene, thus elevating claudin 2 protein production threefold. IL-13 did not have a significant effect on the expression of occludin, claudin 1 and claudin 4.

In CD, interferon gamma (IFN-γ) contributes to impairment of epithelial barrier function through disrupting tight junction complexes by causing decreased expression and increased internalization of occludin and ZO-1[68]. The effects of IFN-γ on barrier function may be mediated through 5′ adenosine monophosphate-activated protein kinase (AMPK). AMPK is key in sensing the cell's energy levels, which decrease during inflammation and subsequently increase the demand for AMPK. Scharl *et al*[68] showed that inhibiting AMPK not only reverses its effect on TJs but also its negative effect on trans epithelial resistance. Watson *et al*[69] also showed in T84 cells (model intestinal epithelial cell line) that IFN-γ increased intestinal permeability to large molecules such as *E. coli*-derived lipopolysaccharide. Effects were exerted presumably by decreasing expression of occludin and increasing the expression of claudin 1 but expression of claudin 2 or 3 were not affected[69].

TNF-α can also affect tight junctions and decrease epithelial barrier function by increasing Myosin Light Chain Kinase (MLCK) phosphorylation. Inhibition of MLCK in TNF-α treated epithelial monolayers can acutely restore barrier function[32]. Furthermore, MLCK-activation promotes IL-13 expression and claudin 2 synthesis[70]. TNF-α induced MLCK expression may therefore be a critical mechanism for barrier dysfunction in UC and CD. Ileal MLCK expression is increased in ileal biopsies from patients with CD compared with controls and increased MLCK correlates with disease activity[71].

Animal models of epithelial permeability and inflammation

A recent study of transgenic mice expressing activated MLCK showed increased paracellular permeability without histological inflammation. Further analysis however, found an increase in the absolute numbers of lamina propria $CD4^+$ lymphocytes and a significant redistribution of $CD11c^+$ dendritic cells to the superficial lamina propria as well as polarisation to a Th1 cytokine profile[72]. Despite increased epithelial permeability these mice did not develop intestinal inflammation. However, when crossed with mice that develop spontaneous inflammation an accelerated and exaggerated inflammatory response was seen. This mouse model therefore suggests that barrier loss may not initiate inflammation but accelerate inflammatory responses.

Other studies suggest that tight junction abnormalities and epithelial permeability may precede the increase of inflammatory cytokines. Interleukin-10 (IL-10) blocks IFN-γ induced epithelial permeability and IL-10 knockout mice have increased permeability and spontaneously develop chronic intestinal inflammation[33]. Inhibition of the zonulin receptor (a key receptor in tight junction binding regulation) in IL-10 knockout mice reduced intestinal permeability and attenuated the spontaneous development of colitis[33]. However, increased intestinal permeability in IL-10 knockout mice not only preceded the onset of inflammation but also occurred significantly earlier than any differences in IFN-γ or TNF-α.

A recent study of intestine-specific claudin 7 knockout mice[73] demonstrated increased neutrophil infiltration into the lamina propria and increased mRNA expression of inflammatory cytokines without altered epithelial integrity. Further investigation showed increased epithelial permeability and paracellular absorption of small molecules and increased absorption of a bacterial derived neutrophil chemoattractant in the claudin 7 knockouts. Treatment with antibiotics or exogenous administration of the soluble bacterial derived chemoattractant, abolished and initiated the onset of inflammation respectively. This model

suggests that the loss of claudin 7 enabled increased absorption of soluble bacterial products leading to the development of colonic inflammation.

Ileoanal pouch as a human model for inflammatory bowel disease

Unlike UC and CD, the ileal pouch offers a unique opportunity to study the development of inflammation before disease onset. In patients with UC, increased epithelial expression of the "pore-forming" tight junction claudin 2 was an early event after ileostomy closure and preceded increased IL-6 levels, as well as increased TLR4 and CD40 activation marker expression in patients with mucosal inflammation of the pouch at twelve months following ileostomy closure[54].

THERAPEUTIC OPPORTUNITY IN INFLAMMATORY BOWEL DISEASES

Strategies to manipulate tight junctions and intestinal permeability are likely to have an important role in the future treatment of inflammatory bowel diseases.

Anti-TNF therapy and tight junctions

Anti-TNF therapy is effective in the treatment of Crohn's disease, ulcerative colitis and chronic pouchitis[74-76]. Barrier function is significantly restored following anti-TNF therapy for CD[77,78]. In the study by Zeissig *et al*[78], this was associated with a reduction in epithelial apoptosis but no significant changes in occludin, claudin 1 or claudin 4. However, other claudins including claudin 2 were not assessed. In a study of experimental colitis in mice, both etanercept and infliximab attenuated inflammation induced reductions in ZO-1 and occludin as well as reducing the upregulation of claudin 2[79]. More recently, in epithelial cell lines adalimumab prevented increased phosphorylation of myosin light chain and reversed the TNF induced down regulation of claudins 1 and 4[80].

Short chain fatty acids

In the colon, anaerobic bacterial fermentation of undigested polysaccharides leads to the production of short chain fatty acids, particularly acetic, proprionic and butyric acids. Short chain fatty acids, in particular butyrate are thought to be the principal source of energy for colonocytes and in UC patients colonocytes have demonstrated diminished oxidation of butyrate[81,82]. *In vitro* culture demonstrated butyrate enhanced claudin 1 transcription and enhanced barrier function[83,84]. In colonic epithelial cells treated with butyrate claudin 2 was down regulated[85]. Butyrate might be postulated to have a role in maintaining barrier function *via* tight junction regulation.

Novel compounds and probiotics

Novel compounds that alter epithelial barrier function may be available from nutritional sources. Several plant extracts have been observed to regulate TJ expression. Quercetin, a common flavanoid increase epithelial resistance in Caco-2 cell monolayers by upregulating claudin 4 expression[86]. Berberine, an isoquinolone alkaloid, prevented TNF-α induced claudin 1 disassembly and upregulation of claudin 2 in a cell culture model[87]. Polyunsaturated fatty acids can also have beneficial effects on the assembly and morphology of TJs[88]. Omega-3 and omega 6 polyunsaturated fatty acids up-regulate expression of occludin, reduce permeability and strengthen the epithelial barrier[89] Polyunsaturated fatty acids also reverse the disruptions in TJs caused by proinflammatory cytokines in Caco 2 epithelial cells[90,91] and might play a role in preventing the alteration in the epithelial barrier caused by inflammation or proinflammatory cytokines that could be exploited as a therapeutic target in the treatment of gut inflammation.

Much attention has focused on the effects probiotic bacteria and their products may have on tight junction expression and epithelial barrier function[92]. *In vitro* and animal models have shown Lactobacilli to attenuate epithelial permeability in experimental colitis and to upregulate tight junction expression of ZO-1, occludin and claudin-3[93-95]. VSL#3 (a mixture of eight probiotic strains) prevented the reduction and redistribution of ZO-1 and claudins -1,-3,-4 and -5 in a murine model of colitis. Furthermore, bacterial products may be a source of novel therapies affecting epithelial barrier function. Uncharacterized extracellular proteins secreted by B. longum subsp. infantis, increased the production of ZO-1 and occludin in epithelial cells[96]. Extracellular proteins derived from Lactobacillus rhamnosus GG attenuated reduction in epithelial resistance in an *in vitro* model, preventing the redistribution of tight junction proteins including ZO-1 and occludin in a dose dependent manner[97]. Moreover, *Salmonella* infection increased claudin-2 expression in epithelial cell lines facilitating its invasion. Therefore, blocking claudin-2 as a potential therapeutic target to prevent bacterial invasion has been suggested[98].

Zonulin has been shown to be a key regulator of intestinal permeability through modulation of epithelial tight junctions[99]. A synthetic peptide inhibitor of zonulin known as AT 1001 or Larazotide has undergone clinical studies in the treatment of coeliac disease[100]. In the IL-10 knockout mouse, AT 1001 reduced intestinal permeability and attenuated the development of spontaneous colitis[33]. Future studies are necessary to determine the role these proteins may have in modulating tight junctions and epithelial barrier function in inflammatory bowel diseases.

CONCLUSION

Dysregulation of TJ proteins is involved and may precede the development of IBD. It is probable that they also contribute to the development of IBD-

associated CRC. Recent evidence suggests that a dysregulated expression of TJ proteins may precede the development of intestinal inflammation. However, a number of questions remain unanswered regarding the role of TJs in the aetiology of inflammatory bowel diseases. Significant differences may exist between animal models and human studies regarding TJ expression profiles and further human studies are necessary to elucidate the role of TJs in IBD aetiology or acceleration of aberrant inflammatory responses.

Claudin 2 appears to be upregulated in UC, CD and pouchitis and some studies also suggest elevated claudin 2 to be present in quiescent disease. However, there is little consensus regarding the up- or down regulation of the other claudin isoforms in these conditions. This may be explained to some extent by methodological differences and heterogeneity of patients and sampling with regard to disease activity and history. Further evaluation of the patterns of expression of claudins in active and inactive IBD patients should help to elucidate their contribution to disease, but longitudinal studies are also necessary. Future studies should evaluate therapeutic approaches that manipulate TJs, restoring epithelial barrier integrity, for the treatment of active inflammatory bowel diseases, maintenance of remission and prevention of onset of inflammation in the gut.

REFERENCES

1 **Krug SM**, Schulzke JD, Fromm M. Tight junction, selective permeability, and related diseases. *Semin Cell Dev Biol* 2014; **36**: 166-176 [PMID: 25220018 DOI: 10.1016/j.semcdb.2014.09.002]

2 **Salim SY**, Söderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflamm Bowel Dis* 2011; **17**: 362-381 [PMID: 20725949 DOI: 10.1002/ibd.21403]

3 **Edelblum KL**, Turner JR. The tight junction in inflammatory disease: communication breakdown. *Curr Opin Pharmacol* 2009; **9**: 715-720 [PMID: 19632896 DOI: 10.1016/j.coph.2009.06.022]

4 **Groschwitz KR**, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009; **124**: 3-20; quiz 21-22 [PMID: 19560575 DOI: 10.1016/j.jaci.2009.05.038]

5 **Shen L**, Weber CR, Turner JR. The tight junction protein complex undergoes rapid and continuous molecular remodeling at steady state. *J Cell Biol* 2008; **181**: 683-695 [PMID: 18474622 DOI: 10.1083/jcb.200711165]

6 **Schulzke JD**, Ploeger S, Amasheh M, Fromm A, Zeissig S, Troeger H, Richter J, Bojarski C, Schumann M, Fromm M. Epithelial tight junctions in intestinal inflammation. *Ann N Y Acad Sci* 2009; **1165**: 294-300 [PMID: 19538319 DOI: 10.1111/j.1749-6632.2009.04062.x]

7 **Amasheh S**, Fromm M, Günzel D. Claudins of intestine and nephron - a correlation of molecular tight junction structure and barrier function. *Acta Physiol* (Oxf) 2011; **201**: 133-140 [PMID: 20518752 DOI: 10.1111/j.1748-1716.2010.02148.x]

8 **Rahner C**, Mitic LL, Anderson JM. Heterogeneity in expression and subcellular localization of claudins 2, 3, 4, and 5 in the rat liver, pancreas, and gut. *Gastroenterology* 2001; **120**: 411-422 [PMID: 11159882]

9 **Escaffit F**, Boudreau F, Beaulieu JF. Differential expression of claudin-2 along the human intestine: Implication of GATA-4 in the maintenance of claudin-2 in differentiating cells. *J Cell Physiol* 2005; **203**: 15-26 [PMID: 15389642 DOI: 10.1002/jcp.20189]

10 **Hartsock A**, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta* 2008; **1778**: 660-669 [PMID: 17854762 DOI: 10.1016/j.bbamem.2007.07.012]

11 **Bruewer M**, Samarin S, Nusrat A. Inflammatory bowel disease and the apical junctional complex. *Ann N Y Acad Sci* 2006; **1072**: 242-252 [PMID: 17057204 DOI: 10.1196/annals.1326.017]

12 **Schneeberger EE**, Lynch RD. Structure, function, and regulation of cellular tight junctions. *Am J Physiol* 1992; **262**: L647-L661 [PMID: 1616050]

13 **Turksen K**, Troy TC. Barriers built on claudins. *J Cell Sci* 2004; **117**: 2435-2447 [PMID: 15159449 DOI: 10.1242/jcs.01235]

14 **Morita K**, Furuse M, Fujimoto K, Tsukita S. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc Natl Acad Sci USA* 1999; **96**: 511-516 [PMID: 9892664]

15 **Amasheh S**, Meiri N, Gitter AH, Schöneberg T, Mankertz J, Schulzke JD, Fromm M. Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J Cell Sci* 2002; **115**: 4969-4976 [PMID: 12432083]

16 **Rosenthal R**, Milatz S, Krug SM, Oelrich B, Schulzke JD, Amasheh S, Günzel D, Fromm M. Claudin-2, a component of the tight junction, forms a paracellular water channel. *J Cell Sci* 2010; **123**: 1913-1921 [PMID: 20460438 DOI: 10.1242/jcs.060665]

17 **Furuse M**, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, Noda T, Kubo A, Tsukita S. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol* 2002; **156**: 1099-1111 [PMID: 11889141 DOI: 10.1083/jcb.200110122]

18 **Milatz S**, Krug SM, Rosenthal R, Günzel D, Müller D, Schulzke JD, Amasheh S, Fromm M. Claudin-3 acts as a sealing component of the tight junction for ions of either charge and uncharged solutes. *Biochim Biophys Acta* 2010; **1798**: 2048-2057 [PMID: 20655293 DOI: 10.1016/j.bbamem.2010.07.014]

19 **Van Itallie C**, Rahner C, Anderson JM. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. *J Clin Invest* 2001; **107**: 1319-1327 [PMID: 11375422 DOI: 10.1172/jci12464]

20 **Amasheh S**, Schmidt T, Mahn M, Florian P, Mankertz J, Tavalali S, Gitter AH, Schulzke JD, Fromm M. Contribution of claudin-5 to barrier properties in tight junctions of epithelial cells. *Cell Tissue Res* 2005; **321**: 89-96 [PMID: 16158492]

21 **Angelow S**, Kim KJ, Yu AS. Claudin-8 modulates paracellular permeability to acidic and basic ions in MDCK II cells. *J Physiol* 2006; **571**: 15-26 [PMID: 16322055 DOI: 10.1113/jphysiol.2005.099135]

22 **Angelow S**, Yu AS. Claudins and paracellular transport: an update. *Curr Opin Nephrol Hypertens* 2007; **16**: 459-464 [PMID: 17693762 DOI: 10.1097/MNH.0b013e32820ac97d]

23 **Angelow S**, Ahlstrom R, Yu AS. Biology of claudins. *Am J Physiol Renal Physiol* 2008; **295**: F867-F876 [PMID: 18480174 DOI: 10.1152/ajprenal.90264.2008]

24 **Baumgart DC**, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007; **369**: 1627-1640 [PMID: 17499605 DOI: 10.1016/s0140-6736(07)60750-8]

25 **Teshima CW**, Dieleman LA, Meddings JB. Abnormal intestinal permeability in Crohn's disease pathogenesis. *Ann N Y Acad Sci* 2012; **1258**: 159-165 [PMID: 22731729 DOI: 10.1111/j.1749-6632.2012.06612.x]

26 **Weber CR**, Turner JR. Inflammatory bowel disease: is it really just another break in the wall? *Gut* 2007; **56**: 6-8 [PMID: 17172583 DOI: 10.1136/gut.2006.104182]

27 **Clayburgh DR**, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest* 2004; **84**: 282-291 [PMID: 14767487 DOI: 10.1038/labinvest.3700050]

28 **Hollander D**. Crohn's disease--a permeability disorder of the tight junction? *Gut* 1988; **29**: 1621-1624 [PMID: 3065154]

29 **Hollander D**, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn 's disease and their relatives. A possible etiologic factor. *Ann Intern Med* 1986; **105**: 883-885 [PMID: 3777713]

30 **Munkholm P**, Langholz E, Hollander D, Thornberg K, Orholm M, Katz KD, Binder V. Intestinal permeability in patients with Crohn' s disease and ulcerative colitis and their first degree relatives. *Gut* 1994; **35**: 68-72 [PMID: 8307453 DOI: 10.1136/gut.35.1.68]

31 **Heller F**, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Mankertz J, Gitter AH, Bürgel N, Fromm M, Zeitz M, Fuss I, Strober W, Schulzke JD. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology* 2005; **129**: 550-564 [PMID: 16083712 DOI: 10.1016/j.gastro.2005.05.002]

32 **Zolotarevsky Y**, Hecht G, Koutsouris A, Gonzalez DE, Quan C, Tom J, Mrsny RJ, Turner JR. A membrane-permeant peptide that inhibits MLC kinase restores barrier function in in vitro models of intestinal disease. *Gastroenterology* 2002; **123**: 163-172 [PMID: 12105845]

33 **Arrieta MC**, Madsen K, Doyle J, Meddings J. Reducing small intestinal permeability attenuates colitis in the IL10 gene-deficient mouse. *Gut* 2009; **58**: 41-48 [PMID: 18829978 DOI: 10.1136/gut.2008.150888]

34 **D'Incà R**, Di Leo V, Corrao G, Martines D, D'Odorico A, Mestriner C, Venturi C, Longo G, Sturniolo GC. Intestinal permeability test as a predictor of clinical course in Crohn's disease. *Am J Gastroenterol* 1999; **94**: 2956-2960 [PMID: 10520851 DOI: 10.1111/j.1572-0241.1999.01444.x]

35 **Peeters M**, Geypens B, Claus D, Nevens H, Ghoos Y, Verbeke G, Baert F, Vermeire S, Vlietinck R, Rutgeerts P. Clustering of increased small intestinal permeability in families with Crohn's disease. *Gastroenterology* 1997; **113**: 802-807 [PMID: 9287971]

36 **Anderson CA**, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A, Lagacé C, Scott R, Amininejad L, Bumpstead S, Baidoo L, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Colombel J-F, Denson LA, De Vos M, Dubinsky M, Edwards C, Ellinghaus D, Fehrmann RSN, Floyd JAB, Florin T, Franchimont D, Franke L, Georges M, Glas J, Glazer NL, Guthery SL, Haritunians T, Hayward NK, Hugot J-P, Jobin G, Laukens D, Lawrance I, Lémann M, Levine A, Libioulle C, Louis E, McGovern DP, Milla M, Montgomery GW, Morley KI, Mowat C, Ng A, Newman W, Ophoff RA, Papi L, Palmieri O, Peyrin-Biroulet L, Panés J, Phillips A, Prescott NJ, Proctor DD, Roberts R, Russell R, Rutgeerts P, Sanderson J, Sans M, Schumm P, Seibold F, Sharma Y, Simms LA, Seielstad M, Steinhart AH, Targan SR, van den Berg LH, Vatn M, Verspaget H, Walters T, Wijmenga C, Wilson DC, Westra H-J, Xavier RJ, Zhao ZZ, Ponsioen CY, Andersen V, Torkvist L, Gazouli M, Anagnou NP, Karlsen TH, Kupcinskas L, Sventoraityte J, Mansfield JC, Kugathasan S, Silverberg MS, Halfvarson J, Rotter JI, Mathew CG, Griffiths AM, Gearry R, Ahmad T, Brant SR, Chamaillard M, Satsangi J, Cho JH, Schreiber S, Daly MJ, Barrett JC, Parkes M, Annese V, Hakonarson H, Radford-Smith G, Duerr RH, Vermeire S, Weersma RK, Rioux JD. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011; **43**: 246-252 [PMID: 21297633 DOI: 10.1038/ng.764]

37 **Barrett JC**, Lee JC, Lees CW, Prescott NJ, Anderson CA, Phillips A, Wesley E, Parnell K, Zhang H, Drummond H, Nimmo ER, Massey D, Blaszczyk K, Elliott T, Cotterill L, Dallal H, Lobo AJ, Mowat C, Sanderson JD, Jewell DP, Newman WG, Edwards C, Ahmad T, Mansfield JC, Satsangi J, Parkes M, Mathew CG, Donnelly P, Peltonen L, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Craddock N, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, McCarthy MI, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Samani N, Trembath RC, Viswanathan AC, Wood N, Spencer CC, Barrett JC, Bellenguez C, Davison D, Freeman C, Strange A, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Perez ML, Potter SC, Ravindrarajah R, Ricketts M, Waller M, Weston P, Widaa S, Whittaker P, Deloukas P, Peltonen L, Mathew CG, Blackwell JM, Brown MA, Corvin A, McCarthy MI, Spencer CC, Attwood AP, Stephens J, Sambrook J, Ouwehand WH, McArdle WL, Ring SM, Strachan DP. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nat Genet* 2009; **41**: 1330-1334 [PMID: 19915572 DOI: 10.1038/ng.483]

38 **Kroesen AJ**, Dullat S, Schulzke JD, Fromm M, Buhr HJ. Permanently increased mucosal permeability in patients with backwash ileitis after ileoanal pouch for ulcerative colitis. *Scand J Gastroenterol* 2008; **43**: 704-711 [PMID: 18569988 DOI: 10.1080/00365520701873206]

39 **Merrett MN**, Soper N, Mortensen N, Jewell DP. Intestinal permeability in the ileal pouch. *Gut* 1996; **39**: 226-230 [PMID: 8991861]

40 **Amasheh S**, Dullat S, Fromm M, Schulzke JD, Buhr HJ, Kroesen AJ. Inflamed pouch mucosa possesses altered tight junctions indicating recurrence of inflammatory bowel disease. *Int J Colorectal Dis* 2009; **24**: 1149-1156 [PMID: 19488769 DOI: 10.1007/s00384-009-0737-8]

41 **Kroesen AJ**, Leistenschneider P, Lehmann K, Ransco C, Dullat S, Blaut M, Schulzke JD, Fromm M, Buhr HJ. Increased bacterial permeation in long-lasting ileoanal pouches. *Inflamm Bowel Dis* 2006; **12**: 736-744 [PMID: 16917229]

42 **Gitter AH**, Wullstein F, Fromm M, Schulzke JD. Epithelial barrier defects in ulcerative colitis: characterization and quantification by electrophysiological imaging. *Gastroenterology* 2001; **121**: 1320-1328 [PMID: 11729111]

43 **Schmitz H**, Barmeyer C, Fromm M, Runkel N, Foss HD, Bentzel CJ, Riecken EO, Schulzke JD. Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. *Gastroenterology* 1999; **116**: 301-309 [PMID: 9922310]

44 **Prasad S**, Mingrino R, Kaukinen K, Hayes KL, Powell RM, MacDonald TT, Collins JE. Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Lab Invest* 2005; **85**: 1139-1162 [PMID: 16007110 DOI: 10.1038/labinvest.3700316]

45 **Oshima T**, Miwa H, Joh T. Changes in the expression of claudins in active ulcerative colitis. *J Gastroenterol Hepatol* 2008; **23** Suppl 2: S146-S150 [PMID: 19120888 DOI: 10.1111/j.1440-1746.2008.05405.x]

46 **Poritz LS**, Harris LR, Kelly AA, Koltun WA. Increase in the tight junction protein claudin-1 in intestinal inflammation. *Dig Dis Sci* 2011; **56**: 2802-2809 [PMID: 21748286 DOI: 10.1007/s10620-011-1688-9]

47 **Zwiers A**, Fuss IJ, Leijen S, Mulder CJ, Kraal G, Bouma G. Increased expression of the tight junction molecule claudin-18 A1 in both experimental colitis and ulcerative colitis. *Inflamm Bowel Dis* 2008; **14**: 1652-1659 [PMID: 18831034 DOI: 10.1002/ibd.20695]

48 **Lameris AL**, Huybers S, Kaukinen K, Mäkelä TH, Bindels RJ, Hoenderop JG, Nevalainen PI. Expression profiling of claudins in the human gastrointestinal tract in health and during inflammatory bowel disease. *Scand J Gastroenterol* 2013; **48**: 58-69 [PMID: 23205909 DOI: 10.3109/00365521.2012.741616]

49 **Wyatt J**, Vogelsang H, Hübl W, Waldhöer T, Lochs H. Intestinal permeability and the prediction of relapse in Crohn's disease. *Lancet* 1993; **341**: 1437-1439 [PMID: 8099141]

50 **Zeissig S**, Bürgel N, Günzel D, Richter J, Mankertz J, Wahnschaffe U, Kroesen AJ, Zeitz M, Fromm M, Schulzke JD. 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 [PMID: 16822808 DOI: 10.1136/gut.2006.094375]

51 **Marin ML**, Greenstein AJ, Geller SA, Gordon RE, Aufses AH. A freeze fracture study of Crohn's disease of the terminal ileum: changes in epithelial tight junction organization. *Am J Gastroenterol* 1983; **78**: 537-547 [PMID: 6613965]

52 **Kucharzik T**, Walsh SV, Chen J, Parkos CA, Nusrat A. Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. *Am J Pathol* 2001; **159**: 2001-2009 [PMID: 11733350 DOI: 10.1016/s0002-9440(10)63051-9]

53 **Das P**, Goswami P, Das TK, Nag T, Sreenivas V, Ahuja V, Panda SK, Gupta SD, Makharia GK. Comparative tight junction protein

expressions in colonic Crohn's disease, ulcerative colitis, and tuberculosis: a new perspective. *Virchows Arch* 2012; **460**: 261-270 [PMID: 22297703 DOI: 10.1007/s00428-012-1195-1]

54 **Landy J**, Al-Hassi HO, Ronde E, English NR, Mann ER, Bernardo D, Ciclitira PJ, Clark SK, Knight SC, Hart AL. Innate immune factors in the development and maintenance of pouchitis. *Inflamm Bowel Dis* 2014; **20**: 1942-1949 [PMID: 25222658 DOI: 10.1097/mib.0000000000000182]

55 **Wu P**, Wu D, Ni C, Ye J, Chen W, Hu G, Wang Z, Wang C, Zhang Z, Xia W, Chen Z, Wang K, Zhang T, Xu J, Han Y, Zhang T, Wu X, Wang J, Gong W, Zheng S, Qiu F, Yan J, Huang J. γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity* 2014; **40**: 785-800 [PMID: 24816404 DOI: 10.1016/j.immuni.2014.03.013]

56 **Grivennikov SI**, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* 2012; **491**: 254-258 [PMID: 23034650 DOI: 10.1038/nature11465]

57 **Dejea CM**, Wick EC, Hechenbleikner EM, White JR, Mark Welch JL, Rossetti BJ, Peterson SN, Snesrud EC, Borisy GG, Lazarev M, Stein E, Vadivelu J, Roslani AC, Malik AA, Wanyiri JW, Goh KL, Thevambiga I, Fu K, Wan F, Llosa N, Housseau F, Romans K, Wu X, McAllister FM, Wu S, Vogelstein B, Kinzler KW, Pardoll DM, Sears CL. Microbiota organization is a distinct feature of proximal colorectal cancers. *Proc Natl Acad Sci USA* 2014; **111**: 18321-18326 [PMID: 25489084 DOI: 10.1073/pnas.1406199111]

58 **Turksen K**, Troy TC. Junctions gone bad: claudins and loss of the barrier in cancer. *Biochim Biophys Acta* 2011; **1816**: 73-79 [PMID: 21515339 DOI: 10.1016/j.bbcan.2011.04.001]

59 **Dhawan P**, Ahmad R, Chaturvedi R, Smith JJ, Midha R, Mittal MK, Krishnan M, Chen X, Eschrich S, Yeatman TJ, Harris RC, Washington MK, Wilson KT, Beauchamp RD, Singh AB. Claudin-2 expression increases tumorigenicity of colon cancer cells: role of epidermal growth factor receptor activation. *Oncogene* 2011; **30**: 3234-3247 [PMID: 21383692 DOI: 10.1038/onc.2011.43]

60 **Mullin JM**. Potential interplay between luminal growth factors and increased tight junction permeability in epithelial carcinogenesis. *J Exp Zool* 1997; **279**: 484-489 [PMID: 9392870]

61 **Ren J**, Hamada J, Takeichi N, Fujikawa S, Kobayashi H. Ultrastructural differences in junctional intercellular communication between highly and weakly metastatic clones derived from rat mammary carcinoma. *Cancer Res* 1990; **50**: 358-362 [PMID: 2295075]

62 **Ersoz S**, Mungan S, Cobanoglu U, Turgutalp H, Ozoran Y. Prognostic importance of Claudin-1 and Claudin-4 expression in colon carcinomas. *Pathol Res Pract* 2011; **207**: 285-289 [PMID: 21493012 DOI: 10.1016/j.prp.2011.01.011]

63 **Webb PG**, Spillman MA, Baumgartner HK. Claudins play a role in normal and tumor cell motility. *BMC Cell Biol* 2013; **14**: 19 [PMID: 23521713 DOI: 10.1186/1471-2121-14-19]

64 **Mees ST**, Mennigen R, Spieker T, Rijcken E, Senninger N, Haier J, Bruewer M. Expression of tight and adherens junction proteins in ulcerative colitis associated colorectal carcinoma: upregulation of claudin-1, claudin-3, claudin-4, and beta-catenin. *Int J Colorectal Dis* 2009; **24**: 361-368 [PMID: 19184060 DOI: 10.1007/s00384-009-0653-y]

65 **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**: 1110-1120 [PMID: 18711353 DOI: 10.1038/labinvest.2008.78]

66 **Kinugasa T**, Akagi Y, Yoshida T, Ryu Y, Shiratuchi I, Ishibashi N, Shirouzu K. Increased claudin-1 protein expression contributes to tumorigenesis in ulcerative colitis-associated colorectal cancer. *Anticancer Res* 2010; **30**: 3181-3186 [PMID: 20871038]

67 **Gupta RB**, Harpaz N, Itzkowitz S, Hossain S, Matula S, Kornbluth A, Bodian C, Ullman T. Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* 2007; **133**: 1099-1105; quiz 1340-1341 [PMID: 17919486 DOI: 10.1053/j.gastro.2007.08.001]

68 **Scharl M**, Paul G, Barrett KE, McCole DF. AMP-activated protein kinase mediates the interferon-gamma-induced decrease in intestinal epithelial barrier function. *J Biol Chem* 2009; **284**: 27952-27963 [PMID: 19654324 DOI: 10.1074/jbc.M109.046292]

69 **Watson CJ**, Hoare CJ, Garrod DR, Carlson GL, Warhurst G. Interferon-gamma selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. *J Cell Sci* 2005; **118**: 5221-5230 [PMID: 16249235 DOI: 10.1242/jcs.02630]

70 **Weber CR**, Raleigh DR, Su L, Shen L, Sullivan EA, Wang Y, Turner JR. Epithelial myosin light chain kinase activation induces mucosal interleukin-13 expression to alter tight junction ion selectivity. *J Biol Chem* 2010; **285**: 12037-12046 [PMID: 20177070 DOI: 10.1074/jbc.M109.064808]

71 **Blair SA**, Kane SV, Clayburgh DR, Turner JR. Epithelial myosin light chain kinase expression and activity are upregulated in inflammatory bowel disease. *Lab Invest* 2006; **86**: 191-201 [PMID: 16402035 DOI: 10.1038/labinvest.3700373]

72 **Su L**, Shen L, Clayburgh DR, Nalle SC, Sullivan EA, Meddings JB, Abraham C, Turner JR. Targeted epithelial tight junction dysfunction causes immune activation and contributes to development of experimental colitis. *Gastroenterology* 2009; **136**: 551-563 [PMID: 19027740 DOI: 10.1053/j.gastro.2008.10.081]

73 **Tanaka H**, Takechi M, Kiyonari H, Shioi G, Tamura A, Tsukita S. Intestinal deletion of Claudin-7 enhances paracellular organic solute flux and initiates colonic inflammation in mice. *Gut* 2015; **64**: 1529-1538 [PMID: 25691495 DOI: 10.1136/gutjnl-2014-308419]

74 **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549 [PMID: 12047962 DOI: 10.1016/s0140-6736(02)08512-4]

75 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]

76 **Barreiro-de Acosta M**, García-Bosch O, Souto R, Mañosa M, Miranda J, García-Sanchez V, Gordillo J, Chacon S, Loras C, Carpio D, Maroto N, Menchén L, Rojas-Feria M, Sierra M, Villoria A, Marin-Jimenez I. Efficacy of infliximab rescue therapy in patients with chronic refractory pouchitis: a multicenter study. *Inflamm Bowel Dis* 2012; **18**: 812-817 [PMID: 21826765]

77 **Suenaert P**, Bulteel V, Lemmens L, Noman M, Geypens B, Van Assche G, Geboes K, Ceuppens JL, Rutgeerts P. Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. *Am J Gastroenterol* 2002; **97**: 2000-2004 [PMID: 12190167 DOI: 10.1111/j.1572-0241.2002.05914.x]

78 **Zeissig S**, Bojarski C, Buergel N, Mankertz J, Zeitz M, Fromm M, Schulzke JD. Downregulation of epithelial apoptosis and barrier repair in active Crohn's disease by tumour necrosis factor alpha antibody treatment. *Gut* 2004; **53**: 1295-1302 [PMID: 15306588 DOI: 10.1136/gut.2003.036632]

79 **Fries W**, Muja C, Crisafulli C, Cuzzocrea S, Mazzon E. Dynamics of enterocyte tight junctions: effect of experimental colitis and two different anti-TNF strategies. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G938-G947 [PMID: 18258792 DOI: 10.1152/ajpgi.00469.2007]

80 **Fischer A**, Gluth M, Pape UF, Wiedenmann B, Theuring F, Baumgart DC. Adalimumab prevents barrier dysfunction and antagonizes distinct effects of TNF-α on tight junction proteins and signaling pathways in intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G970-G979 [PMID: 23538493 DOI: 10.1152/ajpgi.00183.2012]

81 **Roediger WE**. The colonic epithelium in ulcerative colitis: an energy-deficiency disease? *Lancet* 1980; **2**: 712-715 [PMID:

6106826]
82 **Thibault R**, Blachier F, Darcy-Vrillon B, de Coppet P, Bourreille A, Segain JP. Butyrate utilization by the colonic mucosa in inflammatory bowel diseases: a transport deficiency. *Inflamm Bowel Dis* 2010; **16**: 684-695 [PMID: 19774643 DOI: 10.1002/ibd.21108]
83 **Chapman MA**, Grahn MF, Hutton M, Williams NS. Butyrate metabolism in the terminal ileal mucosa of patients with ulcerative colitis. *Br J Surg* 1995; **82**: 36-38 [PMID: 7881952]
84 **Wang HB**, Wang PY, Wang X, Wan YL, Liu YC. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig Dis Sci* 2012; **57**: 3126-3135 [PMID: 22684624 DOI: 10.1007/s10620-012-2259-4]
85 **Daly K**, Shirazi-Beechey SP. Microarray analysis of butyrate regulated genes in colonic epithelial cells. *DNA Cell Biol* 2006; **25**: 49-62 [PMID: 16405400 DOI: 10.1089/dna.2006.25.49]
86 **Amasheh M**, Schlichter S, Amasheh S, Mankertz J, Zeitz M, Fromm M, Schulzke JD. Quercetin enhances epithelial barrier function and increases claudin-4 expression in Caco-2 cells. *J Nutr* 2008; **138**: 1067-1073 [PMID: 18492835]
87 **Amasheh M**, Fromm A, Krug SM, Amasheh S, Andres S, Zeitz M, Fromm M, Schulzke JD. TNFalpha-induced and berberine-antagonized tight junction barrier impairment via tyrosine kinase, Akt and NFkappaB signaling. *J Cell Sci* 2010; **123**: 4145-4155 [PMID: 21062898 DOI: 10.1242/jcs.070896]
88 **Ulluwishewa D**, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr* 2011; **141**: 769-776 [PMID: 21430248 DOI: 10.3945/jn.110.135657]
89 **Li Q**, Zhang Q, Zhang M, Wang C, Zhu Z, Li N, Li J. Effect of n-3 polyunsaturated fatty acids on membrane microdomain localization of tight junction proteins in experimental colitis. *FEBS J* 2008; **275**: 411-420 [PMID: 18167140 DOI: 10.1111/j.1742-4658.2007.06210.x]
90 **Li Q**, Zhang Q, Wang M, Zhao S, Xu G, Li J. n-3 polyunsaturated fatty acids prevent disruption of epithelial barrier function induced by proinflammatory cytokines. *Mol Immunol* 2008; **45**: 1356-1365 [PMID: 17936906 DOI: 10.1016/j.molimm.2007.09.003]
91 **Amasheh M**, Andres S, Amasheh S, Fromm M, Schulzke JD. Barrier effects of nutritional factors. *Ann N Y Acad Sci* 2009; **1165**: 267-273 [PMID: 19538315 DOI: 10.1111/j.1749-6632.2009.04063.x]
92 **Bergmann KR**, Liu SX, Tian R, Kushnir A, Turner JR, Li HL, Chou PM, Weber CR, De Plaen IG. Bifidobacteria stabilize claudins at tight junctions and prevent intestinal barrier dysfunction in mouse necrotizing enterocolitis. *Am J Pathol* 2013; **182**: 1595-1606 [PMID: 23470164 DOI: 10.1016/j.ajpath.2013.01.013]
93 **Anderson RC**, Cookson AL, McNabb WC, Park Z, McCann MJ, Kelly WJ, Roy NC. Lactobacillus plantarum MB452 enhances the function of the intestinal barrier by increasing the expression levels of genes involved in tight junction formation. *BMC Microbiol* 2010; **10**: 316 [PMID: 21143932 DOI: 10.1186/1471-2180-10-316]
94 **Liu ZH**, Shen TY, Zhang P, Ma YL, Moyer MP, Qin HL. Protective effects of Lactobacillus plantarum against epithelial barrier dysfunction of human colon cell line NCM460. *World J Gastroenterol* 2010; **16**: 5759-5765 [PMID: 21128328 DOI: 10.3748/wjg.v16.i45.5759]
95 **Patel RM**, Myers LS, Kurundkar AR, Maheshwari A, Nusrat A, Lin PW. Probiotic bacteria induce maturation of intestinal claudin 3 expression and barrier function. *Am J Pathol* 2012; **180**: 626-635 [PMID: 22155109 DOI: 10.1016/j.ajpath.2011.10.025]
96 **Ewaschuk JB**, Diaz H, Meddings L, Diederichs B, Dmytrash A, Backer J, Looijer-van Langen M, Madsen KL. Secreted bioactive factors from Bifidobacterium infantis enhance epithelial cell barrier function. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G1025-G1034 [PMID: 18787064 DOI: 10.1152/ajpgi.90227.2008]
97 **Seth A**, Yan F, Polk DB, Rao RK. Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G1060-G1069 [PMID: 18292183 DOI: 10.1152/ajpgi.00202.2007]
98 **Zhang YG**, Wu S, Xia Y, Sun J. Salmonella infection upregulates the leaky protein claudin-2 in intestinal epithelial cells. *PLoS One* 2013; **8**: e58606 [PMID: 23505542 DOI: 10.1371/journal.pone.0058606]
99 **Fasano A**. Zonulin, regulation of tight junctions, and autoimmune diseases. *Ann N Y Acad Sci* 2012; **1258**: 25-33 [PMID: 22731712 DOI: 10.1111/j.1749-6632.2012.06538.x]
100 **Paterson BM**, Lammers KM, Arrieta MC, Fasano A, Meddings JB. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study. *Aliment Pharmacol Ther* 2007; **26**: 757-766 [PMID: 17697209 DOI: 10.1111/j.1365-2036.2007.03413.x]
101 **Denizot J**, Sivignon A, Barreau F, Darcha C, Chan HF, Stanners CP, Hofman P, Darfeuille-Michaud A, Barnich N. Adherent-invasive Escherichia coli induce claudin-2 expression and barrier defect in CEABAC10 mice and Crohn's disease patients. *Inflamm Bowel Dis* 2012; **18**: 294-304 [PMID: 21688348 DOI: 10.1002/ibd.21787]
102 **Feldman GJ**, Mullin JM, Ryan MP. Occludin: structure, function and regulation. *Adv Drug Deliv Rev* 2005; **57**: 883-917 [PMID: 15820558 DOI: 10.1016/j.addr.2005.01.009]
103 **Umeda K**, Matsui T, Nakayama M, Furuse K, Sasaki H, Furuse M, Tsukita S. Establishment and characterization of cultured epithelial cells lacking expression of ZO-1. *J Biol Chem* 2004; **279**: 44785-44794 [PMID: 15292177 DOI: 10.1074/jbc.M406563200]

P- Reviewer: Abdulnour-Nakhoul S **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Wang CH

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327