Molecular regulation of the intestinal epithelial barrier: implication in human diseases

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1. ABSTRACT

Intestinal barrier dysfunction is implicated in the development of various clinical diseases. While the study of intestinal barrier function has traditionally emphasized the impact of intestinal microflora and bacteria, the rapid development of molecular and cellular techniques has helped the recent transition of the field to the molecular regulation of the intestinal epithelial barrier. In this review, we summarized several aspects of recent progress on the molecular regulation of the intestinal epithelial barrier, ranging from the extrinsic factors such as probiotics, intrinsic protein effectors including the tight junction proteins, intestinal alkaline phosphatase and protein phosphatase 2A, to intestinal cell subsets such as intestinal intraepithelial lymphocytes and intestinal stem cells. Further investigations into the detailed mechanisms underlying the molecular regulation of the intestinal epithelial barrier will enable our manipulation of the factors and cell subsets involved to develop effective approaches to treat intestinal barrier dysfunction associated diseases.

2. INTRODUCTION

Epithelial mucosal barrier is the most important intestinal barrier, which consists of complete intestinal epithelial cells (IECs) and the connection between the adjacent IECs (1). Epithelial mucosal barrier also regulates the transepithelial transport of water and solution. Human gut contains 500-1000 different kinds of microorganisms, with the highest bacterial load of up to 10^{12} bacteria per gram feces in the colon particularly (2-3). Clinically, the intestinal barrier dysfunction acts as a "spark" in the pathological process of traumatic organ dysfunction (4). Upon the body is subjected to the "first blow" by trauma, the innate immune system and neutrophil system can be activated with the release of a large number of cytokines, followed by increased intestinal permeability, local ischemic edema, and even shock. Inappropriate fluid resuscitation or surgery may bring the "second attack", leading to ischemia and reperfusion injury and the occurrence of systemic inflammatory response syndrome (SIRS). Furthermore, increased intestinal permeability will
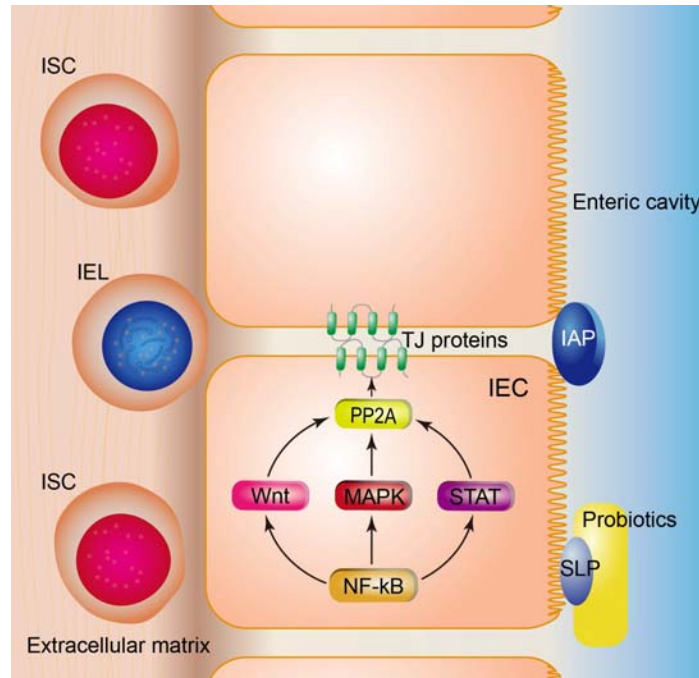
Figure 1. Location of the moleculars related to the intestinal barrier function.

promote intestinal bacterial translocation which acts as the "third-strike" and results in the acute intestinal barrier distress syndrome (5).

Given the clinical significance of intestinal barrier dysfunction, research on the molecular composition and regulation of intestinal epithelial barrier has gained more attention recently. Furthermore, the contribution of impaired intestinal barrier to a variety of diseases begins to be better appreciated. In this minireview, we summarize recent progress on the understanding of molecular regulation of the intestinal epithelial barrier and the implication in human diseases (Figure. 1).

3. PROBIOTICS HAVE PROTECTIVE EFFECTS ON INTESTINAL BARRIER

It has been established that probiotics have important protective effects on intestinal barrier function (6-8). Probiotics not only stimulate the growth of beneficial bacteria within the intestine, but also inhibit, by a variety of ways, the growth, adhesion and invasion of pathogenic bacteria to keep the flora balance. For example, by adhesion to its target cells IECs, lactobacilli can improve the structural changes of tight junction (TJ) and associated TJ protein distribution in IECs, reduce intestinal permeability, and inhibit apoptosis of IECs, thus preventing intestinal barrier damage caused by pathogenic Escherichia coli (E. coli) (9).

Probiotics have been demonstrated to inhibit NF-κB activation, reduce the number of T lymphocytes within the epithelial tissues, and have anti-inflammatory effects through increasing the expression of anti-inflammatory cytokines such as IL-1 and TGF-β, and decreasing the expression of proinflammatory cytokines such as TNF-α, IL-1β, and INF-γ (10-12). Additionally, lactobacillus has a protective effect on liver barrier dysfunction caused by obstructive jaundice after the common bile duct ligation in rats, perhaps due to the activation of protein kinase C (PKC) in IECs (13-14). Lactobacillus plantarum reduced the expression of adhesion molecules such as Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and intercellular adhesion molecule -1 (ICAM-1), inhibited the secretion of inflammatory cytokines TNF-α and INF-γ, and reduced the intestinal pathological scores of inflammation in IL-10 knockout mice with ulcerative colitis (15).

Interestingly, recent evidence suggests that surface layer protein (SLP) plays a key role in the adhesion of lactobacillus to human IECs and mediating the protective effects on intestinal barrier (16-17). SLP can mediate the adhesion of bacteria to target cells and stimulate intracellular signaling pathways, which then contribute to the maintenance of normal intestinal barrier function. Current research on SLP is focused mainly on the proteins of high molecular weight and the detailed mechanisms of SLP action remain largely elusive. Further studies on this topic will help develop novel SLP-based reagents and approaches to prevent intestinal barrier dysfunction.

4. TJ PLAYS AN IMPORTANT ROLE IN THE MAINTENANCE OF INTESTINAL BARRIER FUNCTION

TJ is commonly found near the lumen side of the adjacent IEC membranes and has a selective barrier function against a varity of gastrointestinal pathogens (18-19). In vitro studies showed that pathogenic E. coli can

damage intercellular TJ and impair the barrier function of IECs, causing increased secretion of inflammatory mediators such as TNF-α and INF-γ (9). In addition, in IL-10 knockout mice model structural damage to intestinal TJ increased IEC permeability and disrupted intestinal barrier (20-21).

It is well known that TJ is intricately modulated by a myriad of molecules and signaling pathways including the classic PKC, extracellular signal-regulated kinases (ERK), and special ZO-1 associated nucleic acid binding protein (ZONAB), the Pan-nucleoprotein NaCo, and Ras (22-23). Recent studies have demonstrated that the modulation of TJ proteins via phosphorylation has important implications in IEC barrier function. Under normal circumstances, serine and threonine residues but not tyrosine residues of occludin are highly phosphorylated. However, low level of phosphorylation of serine and threonine residues and high level of phosphorylation of tyrosine residues would increase IEC permeability and apoptosis and cause intestinal barrier dysfunction (24). Several kinases including PKC, CK1 and CK2 have been reported to phosphorylate occludin to modulate TJ and intestinal barrier (25). Oxidative stress and other harmful stimuli promote the phosphorylation of ZO-1, leading to reduced trans-epithelium electrical resistance (TER) of IECs and the redistribution of occludin-ZO-1 and E-cadherin-beta-catenin complex in intestinal TJ (26).

Another study found that incubation of human intestinal epithelial cells with a 27-amino acid peptide corresponding to the first loop domain of Claudin interfered with epithelial barrier function by inducing the rearrangement of major TJ proteins, such as occludin, Claudin-1, junctional adhesion molecule-1 (JAM-1), and ZO-1, suggesting the specific interaction of the first loop domain of Claudin-1 with these TJ proteins to assemble into functional TJs. Moreover, the oral administration of the 27-amino acid peptide to rats could increase paracellular gastric permeability (27).

5. PP2A MODULATES IEC APOPTOSIS AND REGENERATION

PP2A has been shown to be negatively correlated with TJ and the maintenance of intestinal barrier (28-29). As a serine/threonine protein phosphatase, PP2A plays an important role in various cellular processes including apoptosis, proliferation and differentiation via the regulation of signaling pathways (30). Emerging evidence suggests that PP2A is crucially involved in the modulation of IEC apoptosis and regeneration to maintain IEC homeostasis.

It is reported that PP2A could inhibit the growth of IEC and promote IEC apoptosis in mice, which may be attributed to the dephosphorylation of eukaryotic initiation factor 4E (eIF-4E) binding protein 1 (4E-BP1) (31). The addition of PP2A inhibitors can significantly alleviate the intestinal barrier function damage induced by oxidative stress in Caco-2 cells, including increasing the IEC resistance, reducing epithelial permeability, and inducing

ZO-1 expression and re-distribution of other TJ associated proteins (28). Moreover, PP2A can inhibit Akt activity in IECs via the dephosphorylation of Akt active sites Ser308 and Ser473, thus blocking the Ras-PI3K-Akt anti-apoptotic pathway (32). Peroxynitrite can stimulate p38 in IECs to activate PP2A, which then inhibits Akt activity and induces apoptosis of IEC (32). PP2A also participates in the regulation of intracellular STAT3 phosphorylation in IECs, thus modulating its subcellular distribution and binding with DNA. PP2A inhibitor can induce STAT3 phosphorylation at serine and threonine residues and modulate intestinal mucosa repair (33). In addition, deletion or inactivation of STAT5b in IECs could activate Toll-like receptor 2 (TLR2) and NF-κB, leading to increased apoptosis and IEC damage and sever intestinal mucosal immune reaction and inflammatory responses (34). PP2A is the major negative regulatory protein in the Wnt signal pathway, which is crucial for the proliferation and differentiation of IEC. In addition, PP2A activity can increase the dephosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177 and inhibit its activity, thereby reducing NO synthesis and regulating the apoptosis of IEC (35). PP2A also promotes apoptosis of IEC by regulating cyclin dependent kinase (Cdk1) and cell division cycle protein (Cdc25C) (36).

Taken together, PP2A is crucially involved in the maintenance of intestinal barrier function by regulating IEC apoptosis and regeneration, thus represents a potential target for the protection IEC from damage.

6. INTESTINAL ALKALINE PHOSPHATASE PROTECTS THE INTESTINAL BARRIER FUNCTION BY INHIBITING INFLAMMATION

Alkaline phosphatase is distributed in various organs in the body (37). Intestinal alkaline phosphatase (IAP) is a traditional marker of intestinal maturation, but its physiological function in the intestine is not completely understood (38). IAP is located on the brush border of villi in intestinal epithelial, and can be secreted into the intestine in the form of surfactant-like particles. IAP is regulated by endotoxin of the normal intestinal flora (39). When intestinal flora is established in human body, sharp increase in IAP activity may be related to intestinal digestion and absorption, with significance to the maintenance of normal intestinal barrier function (40). Although IAP deficient mice had no obvious digestive dysfunction, IAP has been shown to alleviate the intestinal inflammation and maintain normal bowel function (41-42). Furthermore, IAP deficient zebrafish demonstrated increased sensitivity to lipopolysaccharide (LPS), which could induce neutrophil aggregation and intestinal inflammation through MyD88 and tumor necrosis factor receptor (TNFR), while under sterile conditions, neutrophils in the gut of IAP deficient zebrafish were deficient (38).

Excessive accumulation of LPS and inflammatory factors could inhibit the generation of IAP, resulting in more severe inflammation and anti-inflammatory response syndrome (43). Intestinal pH also

modulates the activity of IAP. HCO_3^- can increase the hydrolytic activity of IAP (44). Normal enteral nutrition has an important role in maintaining the normal function of IAP. In vivo and in vivo experiments showed that IAP inhibited the activity of NF-κB, relieved the toxicity of LPS on IEC, blocked the invasion and displacement of pathogen to the IECs, contributing to normal intestinal barrier function (45). Consistent with this, taking exogenous IAP significantly alleviated the toxicity of LPS in IAP knockout mice (45). Pathological examination revealed that IAP knockout mice treated with glucose anhydride sodium acetate suffered a chronic inflammation of intestinal tissue, however, intestinal inflammation could be relieved after the addition of exogenous IAP to their drinking water (46). Therefore, IAP has potential application in the prevention and treatment of intestinal barrier dysfunction.

7. INTESTINAL INTRAEPITHELIAL LYMPHOCYTES HAVE ANTI-INFECTION AND PROTECTIVE FUNCTION ON THE INTESTINAL BARRIER

Intestinal intraepithelial lymphocytes (IELs) are a heterogeneous group of lymphocytes located between the intestinal epithelial cells or between epithelial cells and basement membrane, and play an important role in the formation of intestinal immune barrier (47). IELs have been divided into two cell types (48). A type of intestinal IELs have similar developmental and antigen recognition characteristics to peripheral lymphoid tissue T cells and include CD8-α-β TCR-α-β IEL; B type of intestinal IELs, which are extremely rare in other parts of the body, are different from normal peripheral T lymphocytes in terms of development, cell phenotype, antigen recognition and biological function, and include CD8-α-α TCR-α-β IEL and TCR-γ-δ IEL (48). While only 1-5% TCR-γ-δ IELs of the total number of T cells were found in the peripheral circulation, the proportion of TCR-γ-δ IELs was as high as 30-40% in the intestine in mice (47). IELs function in immune surveillance and defense not only by exerting the secondary effects on B cells for humoral immunity, but also by secreting a variety of cytokines upon the in vivo or in vitro stimulation, such as IL-2, IL-4, IL-3, IL-5, IL-6, IL-10, INF -γ, TNF-α, and TGF-β (48).

In addition, IELs can express TJ proteins ZO-1 and occluding, and gap junction molecules connexin26, contributing to the protective effects on the intestinal epithelial barrier function (49). For example, TCR-γ-δ IELs enhance anti-injury capacity of the intestinal barrier, whereas activated TCR-α-β CD8-α-β IELs secrete IFN-γ, leading to the damages to intestinal epithelial barrier including reduced resistance and increased permeability of mannitol macromolecules (50). Additional data have shown that IL-15 signaling pathway could upregulate the anti-apoptotic factors Bcl-2 and Bcl-x through reversible phosphorylation of IL-15Rbeta, Jak3, and STAT5 in IELs, thus modulating the inflammatory response and intestinal barrier function (51). These recent studies demonstrate that iIELs play an important role in the maintenance of normal intestinal barrier function and the protection of IECs from

damage. Thus IELs and the related cytokines may open up new possibility of intestinal barrier dysfunction therapy.

8. INTESTINAL STEM CELLS TRANSPLANTATION FOR THE TREATMENT OF INTESTINAL BARRIER DYSFUNCTION

Intestinal Stem Cells (ISCs) are located mainly within the intestinal crypts with the ability of proliferation, self-renewal and differentiation into mature functional IECs (52-56). ISCs also exist in the matrix of the intestinal epithelium and can differentiate into different cell types (57). As pluripotent stem cells, ISCs have attracted attention as a new approach for the treatment of intestinal barrier dysfunction (58). Animal studies have shown that small bowel transplantation of ISCs could reduce the number of pathological T cells and recover the intestinal barrier function through the interaction with the IECs (59-60). Moreover, ISCs could alleviate bleomycin induced inflammation and fibrosis in the small intestine and liver in mice (61). ISCs also inhibited the generation of autoimmune T cells, with no significant effects on the mouse kidney (61).

Clinical case reports and clinical trials showed that in patients with intestinal barrier dysfunction, such as inflammatory bowel disease, the infection was generally under control after intestinal transplantation of ISCs, although serious intestinal infection occurred in very few patients perhaps due to the history of intestinal surgery (59). Phase I clinical trials showed that small intestinal infusion of exogenous ISCs appeared to be without obvious adverse reactions. The infused ISCs stimulated the production of growth factors through paracrine manner, which inhibit the autoimmune response and improve the intestinal and liver function (59). It is reported very recently that Wnt signaling pathway is crucially involved in the differentiation and maturation of ISCs and contributes to the repair of injury to IECs (62). Therefore, the induction and differentiation of transplanted ISCs into IECs may promote the therapy of intestinal barrier dysfunction. Although an increasing body of research has focused on the development of ISCs as a new stem cell technology or tissue engineering technology, basic understanding of the phenotypic and functional characteristics of these cells is urgently needed to optimize their clinical utilization in the treatment of intestinal barrier dysfunction.

9. CONCLUSION

Impaired intestinal barrier is critically implicated in a variety of clinical diseases. Its occurrence, development and regulation are complex and involve both the interaction of host cells with intestinal microenviroment and the modulation of host cells themselves. While the study of intestinal barrier function has traditionally emphasized the impact of intestinal microflora and bacteria, the rapid development of molecular and cellular techniques has helped the recent transition of the field to the molecular regulation of the intestinal epithelial barrier.

In this review, we summarized several aspects of recent progress on the molecular regulation of the intestinal epithelial barrier, ranging from the extrinsic factors such as probiotics, intrinsic protein effectors including the tight junction proteins, intestinal alkaline phosphatase and protein phosphatase 2A, to intestinal cell subsets such as IELs and ISCs. Further investigations into the detailed mechanisms underlying the molecular regulation of the intestinal epithelial barrier will enable our manipulation of the factors and cell subsets involved to develop effective approaches to treat intestinal barrier dysfunction associated diseases.

10. ACKNOWLEDGEMENTS

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