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Biological Functions and Mechanisms of Tight Junctions in Intestinal Mucosal Epithelium[1] Postprint

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Abstract

Tight junctions (TJ) constitute the principal intercellular junctions between intestinal mucosal epithelial cells, playing a crucial role in maintaining epithelial cell polarity and regulating intestinal barrier permeability. TJ form a cellular barrier between mucosal epithelial cells that restricts the movement of solutes and substances. Overall, the structure of TJ can be summarized as a junctional complex composed of transmembrane barrier proteins and surrounding scaffolding proteins. Within this intricate network, numerous associated signaling proteins influence barrier function and broader cellular activities. This article provides a comprehensive review of the biological functions, molecular regulatory mechanisms, and current research status of TJ in intestinal mucosal epithelium.

Full Text

Biological Function and Mechanism of Tight Junctions in Intestinal Mucosal Epithelium

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Abstract: Tight junctions (TJs) are the primary intercellular connections in intestinal mucosal epithelium, playing a crucial role in maintaining epithelial cell polarity and regulating intestinal barrier permeability. TJs form a cellular barrier between mucosal epithelial cells that restricts paracellular movement of solutes and materials. Overall, TJ architecture can be conceptualized as

compartments consisting of transmembrane barrier proteins linked to peripheral scaffolding proteins. Within this complex network, numerous associated signaling proteins affect barrier function and broader cellular activities. This review comprehensively discusses the biological functions, molecular regulatory mechanisms, and current research status of TJs in intestinal mucosal epithelium.

Keywords: intestinal mucosal epithelial cell; tight junction; mucosal barrier; mechanism

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The mucosa constitutes a critical barrier of the intestinal epithelium, formed by connected intestinal epithelial cells (IECs) that regulate trans-epithelial transport of substances such as monosaccharides, amino acids, nucleotides, vitamins, and hormones. Intestinal mucosal epithelial cells feature multiple connection types, including tight junctions (TJ), gap junctions (GJ), adherens junctions (AJ), and desmosomes. Among these, TJs represent the most important intercellular connection, forming a cellular barrier that restricts solute and material movement between mucosal epithelial cells. Numerous proteins have been identified as TJ components, and understanding their organization and interactions is essential for comprehending the biological role of the barrier. The barrier structure can be summarized as protein complexes composed of transmembrane barrier proteins [sealing proteins (claudin) [1], occludin [2], junctional adhesion molecules (JAM) [3], etc.] and peripheral scaffolding proteins [zonula occludens-1 (ZO-1), afadin [4], etc.]. Within this complex network, many associated signaling proteins influence barrier function and broader cellular activities. Transport between mucosal epithelial cells requires paracellular sealing structures to enable directional transport of ions and solutes across cell layers. This sealing structure is formed by TJs, which consist of a series of lateral connections at the most apical junctions between adjacent cells. The TJ structure is shown in Figure 1 [Figure 1: see original paper].

Figure 1 Protein structure of TJ [5]

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1. Biological Characteristics of TJ

1.1 Protein Types

Intestinal mucosal epithelial cells are organized into mucus layer, apical side, and basolateral surface. TJs constitute the most apical structure of the junctional complex in epithelial and endothelial cells, forming barrier structures and paracellular spaces that extend from the apical to basolateral surfaces of the plasma membrane. Studies have shown that TJs contain numerous proteins, and these multi-molecular TJ complex proteins can be divided into three groups: 1) TJ integral proteins (occludin, claudin), which connect apical intercellular spaces and form a regulated permeability barrier; 2) TJ plaque proteins [E-cadherin, ZO-1, JAM-1], most of which express PDZ domains that link integral TJ proteins to

the actin cytoskeleton and serve as adaptor proteins for cytoplasmic molecules associated with cell signaling; and 3) Other proteins [catenins, cingulin, actin, etc.], including regulatory proteins, tumor suppressors, and transcriptional and post-transcriptional factors that directly or indirectly interact with TJ plaque proteins to coordinate diverse functions such as regulating extracellular solute permeability, cell proliferation, cell polarity, and tumor suppression.

1.2 Main Protein Structures

As a barrier, TJs regulate channels for ions and small molecules through the paracellular pathway (barrier function) and restrict lateral diffusion of membrane lipids and proteins between apical and basolateral surfaces to maintain cell polarity (fence function). Among the many proteins comprising TJs, integral proteins are the most important, mainly including occludin and claudin. Occludin is a 64 kDa four-transmembrane protein forming two extracellular loops separated by short cytoplasmic loops, with both amino-terminal and carboxyl-terminal domains located in the cytoplasmic region (Figure 2 [Figure 2: see original paper]). The carboxyl-terminal domain is rich in serine, threonine, and tyrosine residues, making it a target for many proteins and tyrosine kinases. The two extracellular loops have unusual amino acid compositions: the first loop contains a high content (61%) of tyrosine and glycine residues, while the second loop is enriched in tyrosine residues (18%). Very few charged amino acids exist in either loop, and they are predicted to have no net charge at neutral pH. Based on database searches and genomic cloning studies, 24 claudin family members have been identified, with genes predicted to encode 20–27 kDa proteins. Claudin shares no homology with occludin, but similar to occludin, claudin is a four-transmembrane protein with a relatively short cytoplasmic amino- and carboxyl-terminal flank, a first extracellular loop of 53 amino acids, and a second shorter loop of 24 amino acids (Figure 2).

The ZO protein family serves as important scaffolding proteins for TJs, including ZO-1 [6-7], ZO-2, and ZO-3 [8]. These three multi-modular proteins belong to the membrane-associated guanylate kinase-like (MAGUK) family, all structurally containing an amino-terminal PDZ domain, a central Src homology 3 (SH3) domain, a guanylate kinase-like (GUK) domain, and a proline-rich region [9]. These protein-protein interaction domains form stable structures with TJ transmembrane proteins and F-actin cytoplasmic tails, which is critical for strand assembly. In addition to scaffolding functions, ZO proteins have regulatory roles, modulating actomyosin regulators, signaling proteins, transcription factors, and cell polarity [10]. Moreover, under conditions of low cell confluence or junction remodeling, some ZO proteins can shuttle between TJ and the nucleus [11]. These functions enable ZO proteins to act as mechanosensors for dynamic extracellular impacts on TJ, coordinating fundamental cellular processes such as cell polarization, junctional assembly, cell proliferation, and cell differentiation.

Junctional adhesion molecules (JAMs) are glycoproteins belonging to

the immunoglobulin superfamily (IgSF), consisting of two extracellular immunoglobulin-like domains (v-c2 type immunoglobulin domains) [12], one transmembrane region, and a variable cytoplasmic tail. The JAM family comprises three closely related proteins (JAM-A [13-14], JAM-B [15-16], and JAM-C [17-18]) that share 35% amino acid identity and have a short, variable intracellular domain (40–50 residues) at the carboxyl terminus containing a class II PDZ-binding motif [19]. Due to class I and class II PDZ-binding motifs interacting with scaffolding proteins, actin cytoskeleton, and signaling pathways, JAMs and related proteins localize to different cytoplasmic proteins responsible for distinct functional connections. Claudin and claudin-1 are four-transmembrane proteins that do not share sequence homology. Claudin' s first extracellular loop is rich in tyrosine and glycine residues, and both loops contain few charged amino acid residues. In contrast, the amino acid composition of claudin-1' s two extracellular loops varies significantly, resulting in a wide range of isoelectric points. JAM-1 spans the plasma membrane once and has two extracellular immunoglobulin-like domains, with the first amino-terminal loop interacting with homophilic partners on adjacent cells.

Figure 2 Protein structures of occludin, claudin-1 and JAM-1 [1-3]

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2. Function of Major TJ Proteins

Mucosal epithelia form selective barriers between cellular compartments with different fluid and solute compositions, essentially controlled through two pathways: 1) the transcellular pathway, regulated by energy-dependent transporters and asymmetrically distributed apical channels; and 2) the paracellular pathway, where integral TJ proteins span apical intercellular spaces and regulate passive diffusion of ions and small uncharged solutes through paracellular spaces [20]. In addition to serving as a regulated barrier in the paracellular pathway, TJs also function as a fence in the plane of the plasma membrane, helping maintain asymmetric distribution of integral membrane proteins and lipids.

2.1 Claudin Participates in Formation of Ion-Selective Pores

Recent studies using cell membrane-impermeable polyethylene glycol oligomers have demonstrated the existence of restrictive pores within TJs of intestinal cell monolayers, with a radius of 0.43–0.45 nm [21]. Although this data indicates that the TJ barrier is permeable to small uncharged solutes, pores in TJs can distinguish between similarly charged ions, and their permeability depends on ion concentration and environmental pH [22-23]. With few exceptions, most TJs in mucosal epithelia are cation-selective. However, ion permeability (TER) and solute permeability of TJs vary greatly among different tissues and even among cells in the same tissue [24-25]. The presence of only two isoforms of claudin and few charged residues in the two extracellular domains of TJs suggests that claudin alone cannot directly form ion-selective pores. In contrast,

the quantity and form of claudin differ, and the extracellular loops have a wide range of isoelectric points, suggesting that charged amino acid side chains in the extracellular domains of one or more claudins may form selective pores in a given TJ. Experimental evidence indicates that the extracellular domains of TJ proteins are sufficient to form ion-selective pores in the paracellular pathway [26]. However, expression of claudin-4 extracellular domains had greater electrophysiological effects on claudin-2 than overexpression of native claudin-4, suggesting that other domains, including the carboxyl terminus, may play a role in regulating ion selectivity.

Studies on confluent monolayers at steady state under minimally perturbed conditions associated with changes in medium, pH, and temperature have revealed that TJs share biophysical properties with ion channels, including size and charge selectivity, dependence on ion concentration and pH, and the presence of ion competition. These observations suggest [27] that specific claudins appear to determine the ion selectivity of pores in TJs.

2.2 Role of Occludin in TJ

The role of occludin in TJs and its contribution (if any) to the function and/or regulation of ion pores in TJs remain unclear. Overexpression of claudin in Madin-Darby canine kidney (MDCK) cells increases TER but paradoxically increases transepithelial flux of mannitol. Similar functional changes were observed in MDCK cells expressing prostaglandin receptors coupled to G proteins following activation of Ras homolog gene family member A (RhoA)-GTPase, while overexpression of ras-related C3 botulinum toxin substrate 1 (Rac1) or RhoA-GTPase disrupted TJ structure and function. Notably, overexpression of a recently identified TJ-associated Rho-GTPase-specific guanine nucleotide exchange factor (GEF/H1) increased paracellular permeability to hydrophilic solutes without affecting TER. However, the effects of GEF/H1 activity on claudin phosphorylation status and function in TJs were not examined. The possibility that p160ROCK (one of RhoA's key effectors) may regulate claudin phosphorylation and TJ permeability has been examined in endothelial cells. Experimental observations suggest that claudin may be a target of receptor-initiated signals and that claudin phosphorylation can regulate TJ permeability independently of cytoskeletal activity. These observations indicate that occludin may act to coordinate cytoskeletal activity with various signaling pathways necessary for maintaining epithelial cell phenotype.

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3. Regulatory Mechanisms of Intestinal Epithelial TJ

Structural alterations, reduction, or loss of TJs in intestinal mucosal epithelium lead to enlarged intercellular spaces and increased permeability [28], allowing bacteria and endotoxins to enter systemic circulation through intercellular gaps and causing certain intestinal inflammatory diseases such as inflammatory

bowel disease. Although the regulatory mechanisms of these TJs are not fully understood, the following pathways represent basic consensus mechanisms for regulation.

3.1 PLC-Dependent Signaling Pathway

When intestinal epithelial mucosa is stimulated by exogenous or endogenous factors, intestinal mucosal epithelial cells activate PLC through G protein mediation. PLC then hydrolyzes phosphatidyl-inositol 4,5-bisphosphate (PIP2) into 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3), subsequently activating protein kinase C isozymes, calmodulin-dependent kinases, and myosin light chain kinase (MLCK). Changes in PLC activity induce contraction of the perijunctional actin-myosin ring; this contraction and displacement relax TJ structure and alter its function [29], thereby changing intestinal mucosal epithelial permeability. Ethanol and medium-chain fatty acids can also disrupt the perijunctional actin-myosin ring through this mechanism, consequently altering intestinal mucosal epithelial permeability.

3.2 Ca2+-E-cadherin Signaling Pathway

E-cadherin regulates TJs by modulating extracellular Ca2+ concentration. E-cadherin forms five extracellular structural domains containing Ca2+ binding sites. Studies have shown that when extracellular Ca2+ concentration decreases, MLCK can be activated, thereby increasing intestinal mucosal epithelial cell permeability. Experimental evidence indicates that Ca2+ chelators [such as ethylenediaminetetraacetic acid (EDTA)] can increase TJ permeability in intestinal mucosal epithelial cells, possibly by depleting extracellular Ca2+ and reducing Ca2+ availability for E-cadherin [30], leading to disassembly of TJ proteins (occludin and ZO-1) and increased paracellular permeability.

3.3 Tyrosine Kinase-Phosphatase Signaling Pathway

G protein 12 (G 12) belongs to the heterotrimeric G protein family and binds to ZO-1 through the SH3 domain. G 12 activation enhances paracellular permeability in MDCK cells. Meyer et al. [31] found that when G 12 activity was present in MDCK cells, Src autophosphorylation activity increased; simultaneously, -catenin tyrosine phosphorylation also increased. Confocal microscopy revealed disruption of TJ proteins, altered normal distribution of ZO-1 and Na+,K+-ATPase, loss of cell polarity, and increased actin stress fibers. The tyrosine kinase inhibitor genistein and Src-specific inhibitor PP-2 could reverse these changes and prevent increased paracellular permeability. Therefore, G 12 can regulate epithelial cell TJs partially through the Src tyrosine kinase pathway.

3.4 Rho-GTPase Pathway

Rho-GTPase belongs to the G protein family with a molecular mass of 20–30 kDa and can regulate TJ assembly by modulating Rho activity. Studies have found that activation of Rho by Rho-GTPase leads to deposition of ZO-1 and occludin at TJs, thereby maintaining TJ function; inhibition of Rho activity by Rho-GTPase reduces localization of ZO-1 and claudin at TJs. Research has shown that when Rho activity is inhibited while simultaneously depleting ATP, TJ components are substantially lost in transfected cells [32].

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4. Regulatory Factors

4.1 Physicochemical Factors

Studies examining changes in blood-brain barrier permeability and JAM-1 expression in rat cerebral cortex and hippocampus under different power densities of microwave radiation have shown that microwave radiation reduces JAM-1 expression levels in rat brain tissue and increases blood-brain barrier permeability. Other studies have demonstrated that oxygen-glucose deprivation/reoxygenation processes in cultured rat brain microvascular endothelial cells can disrupt intercellular TJs [19].

4.2.1 Fatty Acids

Current research indicates that optimal dietary -linolenic acid/linoleic acid (ALA/LNA) ratios increase expression levels of claudin-3, claudin-b, claudin-c, claudin, and ZO-1 in small intestinal mucosa while decreasing claudin-15a expression in small intestinal mucosa, suggesting that optimal dietary ALA/LNA strengthens TJs in fish intestines. Interestingly, claudin-12 expression levels increased in grass carp intestines at a dietary ALA/LNA ratio of 1.03, which may be related to calcium absorption. Claassen et al. [33] reported that eicosapentaenoic acid and its isomer -linolenic acid (GLA, n-6) can stimulate intestinal calcium absorption in rats. Additionally, enhanced calcium absorption helps regulate claudin-12 expression in mouse intestinal mucosal epithelial cells. These data suggest that increased claudin-12 expression at a dietary ALA/LNA ratio of 1.03 may result from stimulated calcium absorption, which requires further investigation. MLCK plays a crucial role in regulating TJs in mice. Current studies show that MLCK transcriptional abundance was significantly reduced at a ratio of 1.03. Further correlation analysis revealed that claudin-3, claudin-b, claudin-c, claudin, and ZO-1 were negatively correlated with MLCK, while claudin-15a was positively correlated with MLCK mRNA abundance.

4.2.2 Proteins

Hemoglobin can cause disruption of intercellular TJs and increased permeability in endothelial cells, leading to endothelial barrier dysfunction. Hemoglobin

increases intracellular phosphorylated myosin light chain (p-MLC) expression levels, resulting in substantial formation of intracellular filamentous actin and inducing decreased expression of the intercellular TJ protein ZO-1, which may represent an important pathophysiological mechanism for TJ disruption in endothelial cells.

Glucagon-like peptide-2 (GLP-2) is an intestinotrophic hormone. Gradient experiments with GLP-2 were conducted in isolated weaned piglet intestinal tissue blocks by adding different concentrations of GLP-2 and culturing for 72 h. Results demonstrated that appropriate concentrations of GLP-2 promoted expression of ZO-1, occludin, and claudin-1.

Enterotoxin is a 35 kDa protein produced by *Clostridium perfringens* that causes food poisoning in humans. The carboxyl terminus of this protein specifically binds to claudin-3 and claudin-4 [34], while the amino terminus forms pores in the plasma membrane. To specifically bind TJ proteins and avoid plasma membrane damage, the carboxyl terminus of enterotoxin was transfected into L-cells expressing claudin-1, claudin-2, claudin-3, and claudin-4, or confluent MDCK I cells expressing claudin-1 and claudin-4. In L-cell transfectants, the enterotoxin fragment selectively bound to claudin-3 and claudin-4 but not to claudin-1 or claudin-2. Notably, in MDCK I cells, TJs began to disintegrate within 4 h of enterotoxin fragment binding to intestinal mucin peptides, with decreased TER and increased paracellular flux. However, it remains unclear whether the interaction between enterotoxin and claudin-4 leads to disaggregation of proteins in existing TJ strands. Alternatively, since TJ disruption only occurs when enterotoxin is added to the basolateral surface, it is conceivable that interaction between enterotoxin and claudin-4 monomers in the membrane prevents their incorporation into TJ strands, thereby causing TJ disruption.

CagA is a protein produced by *Helicobacter pylori*. When *H. pylori* adheres to the apical junctional complex of epithelial cells, CagA translocates from bacteria into epithelial cells [35]. Notably, CagA appears to target *H. pylori* to intercellular junctions, where enterotoxin interacts with ZO-1 and JAM-1 at bacterial attachment sites. Following this interaction, TJ barrier function is disrupted and cell shape is significantly altered [36]. Although the mechanism causing TJ disruption in *H. pylori* infection remains unclear, perturbation of ZO-1 and JAM-1 may lead to abnormal localization and function of important signaling molecules. If present, the clinical consequences of this interaction may be gastric ulcers or gastric cancer.

TJs are one of the most important connection methods between intestinal mucosal epithelial cells. Their interaction with nutritional factors such as proteins and fatty acids affects TJ protein activity, regulating intestinal epithelial substance transport and permeability. However, the molecular mechanisms of TJ regulation are not yet fully understood. Therefore, in-depth research on gene expression regulation of TJ proteins is of great significance for exploring the regulatory mechanisms of animal intestinal and tissue barriers.

References

[1] FURUSE M, FUJITA K, HIIRAIG T, et al. Claudin-1 and claudin-2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin[J]. *The Journal of Cell Biology*, 2001, 141(7): 1539–1550.

[2] MATSUDA M, KUBO A, FURUSE M, et al. A peculiar internalization of claudins, tight junction-specific adhesion molecules, during intercellular movement of epithelial cells[J]. *The Journal of Cell Biology*, 2004, 117: 1247–1257.

[3] YEAMAN C, GRINDSTAFF K K, NELSON W J. New perspectives on mechanisms involved in generating epithelial cell Polarity[J]. *Physiology Review*, 2005, 79(1): 73–98.

[4] CITI S, DENISENKO N. Phosphorylation of the tight junction protein cingulin and the effects of protein kinase inhibitors and activators in MDCK epithelial cells[J]. *Journal of Cell Science*, 2005, 108: 2917–2926.

[5] TSUKITA S, FURUSE M. Occludin and claudins in tight-junction strands: leading or supporting Players?[J]. *Trends in Cell Biology*, 1999, 9(7): 268–273.

[6] STEVENSON B R, SILICIANO J D, MOOSEKER M S, et al. Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia[J]. *The Journal of Cell Biology*, 2005, 103(3): 755–766.

[7] GUMBINER B, LOWENKOPF T, APATIRA D. Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2005, 88(8): 3460–3464.

[8] HASKINS J, GU L J, WITTCHEN E S, et al. ZO-3, a novel member of the MAGUK protein family found at the tight junction interacts with ZO-1 and occludin[J]. *The Journal of Cell Biology*, 2005, 141: 199–208.

[9] GONZÁLEZ-MARISCAL L, BETANZOS A, ÁVILA-FLORES A. MAGUK proteins: structure and role in the tight junction[J]. *Seminars in Cell & Developmental Biology*, 2000, 11(4): 315–324.

[10] BAUER H, ZWEIMUELLER-MAYER J, STEINBACHER P, et al. The dual role of zonula occludens (ZO) proteins[J]. *Journal of Biomedicine and Biotechnology*, 2010, 2010: 402593.

[11] LOPEZ-BAYGHEN E, JARAMILLOY BE, HUERTA M, et al. TJ Proteins That Make Round Trips to the Nucleus[M]//GONZALEZ-MARISCAL L. *Tight junctions*. [S.l.]: Springer, 2006.

[12] WILLIAMS A F, BARCLAY A N. The immunoglobulin superfamily–domains for cell surface recognition[J]. *Annual Review of Immunology*, 2005, 6(1):

381-405.

[13] MALERGUE F, GALLAND F, MARTIN F, et al. A novel immunoglobulin superfamily junctional molecule expressed by antigen presenting cells, endothelial cells and platelets[J]. *Molecular Immunology*, 2005, 35(17): 1111-1119.

[14] MARTÌN-PADURA I, LOSTAGLIO S, SCHNEEMANN M, et al. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration[J]. *The Journal of Cell Biology*, 2005, 142(1): 117-127.

[15] CUNNINGHAM S A, ARRATE M P, RODRIGUEZ J M, et al. A novel protein with homology junctional adhesion molecule. Characterization leukocyte interactions[J]. *The Journal of Biology Chemistry*, 2000, 275: 34750-34756.

[16] PALMERI D, VAN ZANTE A, HUANG C C, et al. Vascular endothelial junction-associated molecule, a novel member of the immunoglobulin superfamily, is localized to intercellular boundaries of endothelial cells[J]. *The Journal of Biology Chemistry*, 2000, 275(25): 19139-19145.

[17] ARRATE M P, RODRIGUEZ J M, TRAN T M, et al. Cloning of human junctional adhesion molecule 3 (JAM3) and its identification as the JAM2 counter-receptor[J]. *The Journal of Biology Chemistry*, 2001, 276(49): 45826-45832.

[18] AURRAND-LIONS M, DUNCAN L, BALLESTREM C, et al. JAM-2, a novel immunoglobulin superfamily molecule, expressed by endothelial and lymphatic cells[J]. *The Journal of Biology Chemistry*, 2001, 276(4): 2733-2741.

[19] EBNET K, SUZUKI A, OHNO S, et al. Junctional Adhesion Molecules (JAMs): more molecules with dual functions?[J]. *The Journal of Cell Science*, 2004, 117(1): 19-29.

[20] MADARA J L. Intestinal absorptive tight junctions linked cytoskeleton[J]. *American Journal of Physiology: Cell Physiology*, 2005, 253(1): C171-C175.

[21] WATSON C J, ROWLAND M, WARHURST G. Functional modeling of tight junctions in intestinal cell monolayers using polyethylene glycol oligomers[J]. *American Journal of Physiology: Cell Physiology*, 2001, 281(2): 388-397.

[22] TANG V W, GOODENOUGH D A. Paracellular ion channel at tight junction[J]. *Biophysical Journal*, 2003, 84(3): 1660-1673.

[23] YU A S L, ENCK A H, LENCER W I, et al. Claudin-8 expression in Madin-Darby canine kidney cells augments the paracellular barrier to cation permeation[J]. *The Journal of Biology Chemistry*, 2003, 278(19): 17350-17359.

[24] ENCK A H, BERGER U V, YU A S L. Claudin-2 is selectively expressed in proximal nephron mouse kidney[J]. *American Journal Physiology: Renal Physiology*, 2001, 281(5): F966-F974.

[25] KIUCHI-SAISHIN Y, GOTOH S, FURUSE M, et al. Differential expression patterns of claudins, tight junction membrane proteins in mouse nephron segments[J]. *Journal of the American Society of Nephrology*, 2002, 13(4): 875–886.

[26] COLEGIO O R, VAN ITALLIE C M, RAHNER C, et al. Claudin extracellular domains determine paracellular charge selectivity and resistance but not tight junction fibril architecture[J]. *American Journal of Physiology: Cell Physiology*, 2003, 284(6): C1346–C1354.

[27] FURUSE M, FURUSE K, SASAKI H, et al. Conversion of zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells[J]. *The Journal of Biology*, 2001, 153(2): 263–272.

[28] HOLLANDER D, VADHEIM C M, BRETTHOLZ E, et al. Increased intestinal Permeability in patients with Crohn' s disease and their relatives. A possible etiologic factor[J]. *Annals of Internal Medicine*, 2005, 105(6): 883–885.

[29] YAMAGUCHI Y, DALLE-MOLLE E, HARDISON W G. VasoPressin and A23187 stimulate phosphorylaiton of myosin light chain-1 in isolated rat hepatocytes[J]. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, 2005, 261(2): G312–G319.

[30] BEHLENS J, VAKAET L, FRIIS R, et al. Loss of epithelial differentiation and gain of Invasiveness correlates with tyrosine Phosphorylation of the E-cadherin/beta-catenin complex in cells transformed with a temperature-sensitive v-SRC gene[J]. *The Journal of Biology*, 2005, 120(3): 757–766.

[31] MEYER T N, HUNT J, SCHWESINGER C, et al. G 12 regulates epithelial cell junctions through tyrosine kinases[J]. *American Journal Physiology-Cell Physiology*, 2003, 285(5): C1281–C1293.

[32] GOPALAKRISHNAN S, RAMAN N, ATKINSON S J, et al. Rho GTPase signaling regulates tight junction assembly and Protects tight junctions during ATP depletion[J]. *American Journal of Physiology: Cell Physiology*, 2005, 275(3): C798–C809.

[33] CLAASSEN N, COETZER H, STEINMANN C M L, et al. The effect of different n-6n-3 essential fatty acid ratios on calcium balance and bone in rats[J]. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 1995, 53(1): 13–19.

[34] FUJITA K, KATAHIRA J, HORIGUCHI Y, et al. *Clostridium perfringens* enterotoxin binds to the second extracellular loop of claudin-3, a tight junction integral membrane protein[J]. *FEBS Letters*, 2000, 476(3): 258–261.

[35] ODENBREIT S, PÜLS J, SEDLMAIER B, et al. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion[J]. *Science*, 2000, 287(5457): 1497–1500.

[36] AMIEVA M R, VOGELMANN R, COVACCI A, et al. Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA[J]. *Science*, 2005,

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