Tight junction dysfunction and cytoskeletal remodeling in Hirschsprung-associated enterocolitis: A decade of mechanistic insights and therapeutic prospects (Review)

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Abstract. Hirschsprung-associated enterocolitis (HAEC) represents a severe complication of Hirschsprung disease, characterized by intestinal barrier dysfunction and life-threatening inflammation. The present study systematically reviews the updated molecular mechanisms underlying HAEC pathogenesis, with particular focus on the tight junction (TJ) proteins claudin, occludin and zonula occludens protein 1 (ZO-1) and their interactions with the actin cytoskeleton. The present review demonstrates that dysregulation of claudin family members, particularly upregulation of pore-forming claudin-2 and downregulation of barrier-forming claudin-4, disrupts intestinal homeostasis. Occludin undergoes cytokine-mediated endocytosis through myosin light chain kinase (MLCK)/NF-κB signaling, while ZO-1 dysfunction impairs mechanical coupling between TJs and actin filaments. Furthermore, the present review identifies that inflammatory mediators, such as IL-1β, TNF-α and IFN-γ, trigger actin cytoskeleton remodeling via the cofilin phosphorylation cycle and the Rho-associated protein kinase/MLCK pathway, establishing a cycle of barrier breakdown. Importantly, the present review highlights the lipocalin 10/slingshot homologue 1/cofilin axis and TJ-cytoskeleton interactions as mechanistic targets for future intervention in HAEC treatment. These findings provide a comprehensive mechanistic framework for understanding HAEC pathogenesis and offer novel targets for clinical intervention.

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Contents

1. Introduction

Hirschsprung disease (HSCR) is a congenital neurodevelopmental disorder characterized by the absence of enteric ganglion cells in the distal bowel, leading to dysmotility and functional obstruction (1). Its most severe complication, Hirschsprung-associated enterocolitis (HAEC), occurs in 17-50% of patients with HSCR. The preoperative incidence of HAEC peaks at 60% in unscreened cohorts, while postoperative rates range from 25-37% (2,3). In total, >95% of cases occur in children <5 years of age in multicenter studies (4). Asian cohorts report lower postoperative rates (5,6) than Western populations with HRSC (7), with HAEC representing the leading cause of mortality in this population. Clinically, HAEC manifests as fulminant diarrhea progressing to sepsis, driven by mucosal barrier failure, dysbiosis and immune hyperactivation (7,8). Central to this pathophysiology is tight junction (TJ) dysfunction, a 'leaky epithelium' phenotype that facilitates systemic pathogen dissemination and fluid loss (9,10), although its molecular underpinnings remain elusive.

The intestinal epithelial barrier is governed by TJs, which orchestrate selective permeability to balance nutrient absorption and pathogen exclusion (11). TJs are assembled from transmembrane proteins, including claudins, occludin and junctional adhesion molecules, cytoplasmic scaffolds, such as zonula occludens protein 1 (ZO-1) and cingulin, and the actomyosin cytoskeleton comprising filamentous-actin (F-actin) and myosin II (12). Claudins form charge-selective pores, as is the case with claudin-2, or barrier strands, such as claudin-4, while occludin regulates macromolecular transport via lipid raft-dependent endocytosis (13,14). Notably, mechanical coupling between TJ proteins and the actomyosin cytoskeleton is important for maintaining TJ ultrastructural stability (15). Dysregulation of this dynamic assembly system directly precipitates barrier collapse (16).

Studies have shown that in bowel segments lacking ganglion cells, intestinal barrier function is impaired and exhibits decreased expression of ZO-1 and increased expression of claudin-3 (17). These alterations in TJ gene expression may result in increased epithelial permeability, further promoting the development of HAEC. Compared with other HSCR subgroups and patients with anorectal malformations, patients with postoperative HAEC exhibit significantly increased paracellular permeability during radical surgery (18). Additionally, the interaction between TJ proteins and the cytoskeleton, composed of F-actin and myosin, is important for maintaining TJ structure and function (13,15).

M1-polarized pro-inflammatory macrophages secrete IL-1β and TNF-α, downregulating occludin and ZO-1 expression and disrupting actomyosin-TJ coupling (19). This cytokine storm activates the RhoA/Rho-associated protein kinase (ROCK) axis, which induces pathological stress fiber assembly. The formation of these stress fibers dismantles TJ architecture and accelerates luminal toxin influx, propelling HAEC progression toward toxic megacolon (20,21).

The pathogenesis of HAEC involves synergistic defects in tight junction regulation (22) and actomyosin contractility (23). Targeting TJ dynamic assembly or modulating cytoskeletal remodeling may offer precision therapeutic strategies to reverse the 'leaky barrier' phenotype, thereby improving clinical outcomes in HAEC.

2. Claudin

Claudin family and intestinal barrier regulation. The claudin family consists of at least 27 members that serve as important components of TJs, where they determine paracellular ion permeability and charge selectivity. Claudins exhibit tissue-specific expression patterns; for instance, claudin-5 and -6 are predominant in renal podocytes, claudin-2, -4, -8, -12 and -13 in bladder urothelium, claudin-2 to -5 in gastric epithelium and claudin-1 to -19 in murine intestinal epithelium, while human sigmoid colon primarily expresses claudin-1 to -5, -7 and -8. These proteins collectively contribute to barrier formation and function (24).

Functionally, claudins are categorized as either barrier-forming, such as claudin-1, -3 and -4, or pore-forming, such as claudin-2 (16). Disruption of this balance is implicated in intestinal pathologies. For instance, in a benzalkonium chloride (BAC)-induced HSCR model, claudin-3 is upregulated, compromising barrier integrity (17). Conversely, decreased claudin-4 expression is observed in postoperative patients with HAEC (22). Although claudin-1 and -2 have been studied in necrotizing enterocolitis (NEC) (25,26), their roles in HAEC remain to be fully elucidated.

Given their established importance in intestinal barrier regulation, with claudin-1 enhancing barrier tightness, claudin-2 modulating fluid and cation flux (27) and the demonstrated dysregulation of claudin-3 and -4 in HAEC and HSCR, the present study focused on claudin-1 to -4 to systematically investigate their collective and individual roles in HAEC pathogenesis.

Inflammatory signals and reprogramming of the TJ network. The inflammatory microenvironment reprograms the expression of claudins through cytokines, directly disrupting the homeostasis of TJ. For example, IL-1β activates multiple transcription factors, such as NF-κB p50/p65, activating transcription factor-2 and ETS domain-containing protein Elk-1, and through the activation of mitogen-activated protein kinase kinase kinase 1, the catalytic subunit of IKKβ, binds to the minimal promoter region of myosin light chain kinase (MLCK). This synergistically activates the MLCK gene and increases the permeability of TJs (28), thereby promoting intestinal inflammation (Fig. 1) (29). However, regarding the effect of IL-1β on claudins, current research results remain inconsistent. A study by Maria-Ferreira *et al* (30) found that the increase in TJ permeability of Caco-2 cells induced by IL-1β is related to a decrease in claudin-1 expression. By contrast, other studies have shown that IL-1β activates β-catenin via the Wnt signaling pathway or through an MLCK-dependent mechanism, thus downregulating the expression of claudin-3 and leading to an increase in intestinal permeability (31,32).

TNF-α upregulates claudin-2 through the PI3K/Akt signaling pathway (33), enhances the pore effect and causes electrolyte imbalance (Fig. 1). This pore-forming effect is further compounded by a separate pathophysiological process involving the downregulation of barrier-forming claudins. Specifically, in HAEC, the imbalance caused by reduced claudin-1 and -3 expression and elevated claudin-2 expression creates a 'leak-flux' phenotype characteristic of osmotic diarrhea (34). Seemingly conflicting reports on IL-1β activity, e.g. claudin-1 downregulation (30) vs. claudin-3 downregulation (31), reflect spatiotemporal heterogeneity: Claudin-1 dominates in crypt epithelia, while claudin-3 localizes to villus tips (34). The mucosal injury gradient of HAEC may amplify this compartmentalized regulation. In addition, IL-6 and IL-17 enhance the expression of claudin-2 through MAPK signaling (35), while TGF-β regulates the expression of claudin-1 through the MAPK pathway (Fig. 1). Although claudin-1 usually supports the integrity of TJs and increases the baseline transepithelial electrical resistance (TER), inactivation of claudin-2 has been shown to alleviate immune-mediated experimental colitis in mice (36).

Synergistic disruption of the interaction between immunity and TJs. The increase in macrophages and the imbalance of the M1/M2 macrophage ratio can affect intestinal barrier function by influencing TJ proteins. Classically activated M1 macrophages usually infiltrate the intestine during infection

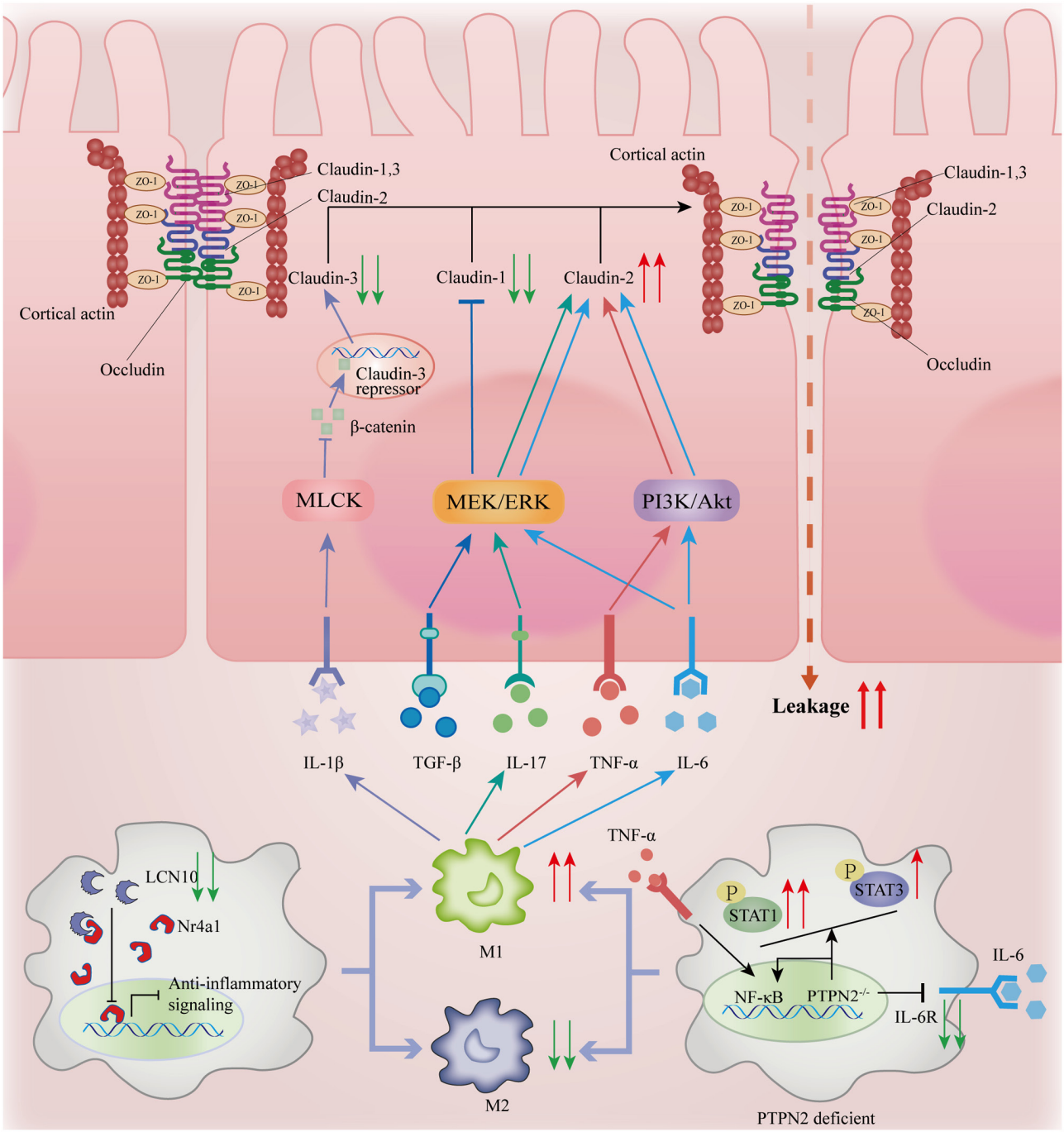

Figure 1. Potential roles of claudins in HAEC. In macrophages with PTPN2 deficiency, the phosphorylation level of STAT1 is elevated and NF-κB activation is increased, which promotes the activation of pro-inflammatory M1 macrophages and leads to the production of high levels of the inflammatory molecules IL-6 and TNF-α. TNF-α further activates NF-κB, driving macrophages to differentiate into the M1 phenotype. Although IL-6 can selectively activate anti-inflammatory M2 macrophages, the expression of IL-6 receptors is significantly reduced in PTPN2-deficient macrophages and the levels of STAT3 are lower compared with STAT1, making these macrophages more likely to differentiate into the M1 subtype. Additionally, the loss or reduction of LCN10 expression in macrophages impairs the nuclear translocation of Nr4a1, leading to an increase in M1 macrophages and a decrease in M2 macrophages. The imbalance between M1 and M2 results in increased levels of pro-inflammatory cytokines such as IL-1β, IL-17, TNF-α and IL-6, while the level of the anti-inflammatory cytokine TGF-β is significantly reduced. IL-1β induces the downregulation of Claudin-3 expression by associating β-catenin with the claudin-3 promoter via the MLCK or Wnt signaling pathways. IL-17 and IL-6 upregulate claudin-2 expression through the MEK/ERK pathway, and TNF-α and IL-6 increase claudin-2 expression via the PI3K/Akt signaling pathway, leading to increased intestinal permeability and intestinal barrier dysfunction, which promotes the development of HAEC. Conversely, TGF-β mediates the expression of claudin-1 through the MEK/ERK signaling pathway, providing protective effects for the intestinal barrier. HAEC, Hirschsprung-associated enterocolitis; PTPN2, protein tyrosine phosphatase non-receptor type 2; Nr4a1, nuclear receptor subfamily 4 group A member 1; MLCK, myosin light chain kinase; LCN10, lipocalin-10; ZO-1, zonula occludens protein 1; P, phosphate group; M1, M1-type macrophage; M2, M2-type macrophage; IL-6R, IL-6 receptor.

or inflammation (37), and an increase in their proportion has been observed during HAEC episodes (38,39). M1 macrophages exacerbate intestinal barrier breakdown through a dual mechanism: i) They secrete TNF-α and IL-6 (Fig. 1), promoting the expression of claudin-2 and inducing the endocytosis and degradation of claudin-4 (40); and ii) M1

macrophages activate heparanase to degrade heparan sulfate within the basement membrane, altering the expression levels of TJ proteins, such as occludin and ZO-1, and disrupting the TJ-extracellular matrix interaction (TJ-ECM) anchoring, thus exacerbating intestinal hyperpermeability (36-38). In macrophages with protein tyrosine phosphatase non-receptor type 2 deficiency, the overactive STAT1/NF-κB signaling pathway drives M1 macrophage polarization and inhibits STAT3-mediated M2 macrophage differentiation by reducing the expression of the IL-6 receptor (35,39). The deficiency of lipocalin 10 (LCN10) further enhances this process by inhibiting the nuclear receptor 4A1 pathway, exacerbating M1 polarization (41) and directly disrupting TJ-cytoskeleton coupling, which leads to barrier leakage (Fig. 1) (42).

Translational potential of targeting the TJ-immunity axis. Based on the aforementioned mechanisms, multimodal intervention strategies show clinical promise. In the BAC pig model, specific small interfering RNA interference of claudin-3 can reduce intestinal permeability and decrease the release of inflammatory factors IL-1β and TNF-α (17). The heparanase inhibitor PG545 restores TJ-ECM anchoring and preclinical trials have shown that the mucosal homeostasis of pediatric patients with HAEC is restored upon treatment (43). The LCN10 protein increases the TER by regulating macrophage polarization (41). These findings not only reveal the multi-level pathogenic mechanisms of HAEC but also provide a theoretical framework for the development of precise therapies targeting the TJ network.

3. Occludin

Molecular architecture and barrier regulation function. Occludin is a four-pass transmembrane protein consisting of 522 amino acids, and its structural features determine its dynamic regulatory function in TJs (44). The two extracellular loops mediate cell-cell adhesion, while the cytoplasmic occludin/ELL (OCEL) domain comprising 107 amino acids engages in interactions with ZO-1, actin and kinases such as MLCK, imparting mechanical stress-response capability to TJs (41,45). Functional studies have demonstrated that occludin specifically regulates the paracellular flux of macromolecules (46) without affecting the fundamental structure of TJs; occludin knockout mice retain intact TJ morphology (43,44), indicating that the core role of occludin is as a 'permeability regulator' rather than a 'structural scaffold.'

Dynamic regulatory mechanisms. The dynamic regulation of occludin in the membrane is a key mechanism in the plasticity of the intestinal barrier. Under inflammation or mechanical stress stimuli, occludin can be directly internalized via vesicle-mediated endocytosis, leading to the dissociation of the TJ supramolecular structure (47). This process is precisely regulated by the lipid raft-scaffolding protein caveolin-1, which has an N-terminus that forms a stable complex with the OCEL domain in the cytoplasmic tail of occludin and modulates its membrane trafficking cycle by altering its phosphorylation pattern (46,48). Inflammatory cytokines, such as TNF-α and IL-1β, trigger hyperphosphorylation of occludin at Ser408 (49). This dysregulation promotes clathrin-mediated endocytic internalization of occludin while disrupting ZO-1 anchoring to the actin cytoskeleton, synergistically increasing paracellular permeability in aganglionic bowel segments (Fig. 2) (50). A study reported by Van Itallie *et al* (51) revealed that knockout of either occludin or caveolin-1 in Madin-Darby canine kidney cells attenuated the disruption of the tight junction barrier induced by inflammatory factors such as TNF-α. *In vivo* experiments support that the absence of caveolin-1 significantly reduces the changes in TJ permeability mediated by TNF-α, suggesting that the pathological function of occludin depends on caveolin-1-mediated lipid raft-signaling microdomain remodeling (51).

Notably, TNF-α triggers a caveolin-1-dependent endocytic cascade by activating the MLCK pathway, a necessary condition for TNF-α regulation of TJ structure and function (50,52). Furthermore, actin depolymerization can exacerbate intestinal barrier breakdown by promoting the clathrin-mediated endocytosis of TJ components, including occludin, and further destabilizing the barrier (53). IL-1β activates microRNA-200c-3p through the MLCK/NF-κB pathway, which binds to the 3'UTR of occludin mRNA and induces its degradation. This degradation results in reduced occludin protein levels, thereby increasing intestinal permeability (Fig. 2) (29). These cooperative mechanisms together form the molecular basis of HAEC (54,55), suggesting that targeting the caveolin-1-occludin axis or the MLCK signaling network could be a potential new strategy for reversing barrier damage.

Pathological mechanisms and targeted intervention strategies. Dysregulated expression of occludin is a common feature of various intestinal diseases. During the active phase of inflammatory bowel disease (IBD), occludin expression is reduced in the colonic epithelium, which negatively correlates with barrier permeability (56). A decrease in occludin protein levels has also been observed in intestinal samples from pediatric patients with NEC (57). In response to this pathological mechanism, ROCK inhibitors can stabilize occludin membrane localization and increase its expression, improving intestinal barrier resistance in the BAC rat model (58).

Contradictory phenotypes and future research framework. Despite occludin inducing epithelial apoptosis through caspase-3 activation (Fig. 2), its deficiency appears to have a protective effect in a mouse model of colitis (59), suggesting that occludin downregulation could be an adaptive stress response. The occludin domain acts as a molecular switch: Its integrity maintains the selective macromolecular barrier function through ZO-1-actin coupling (50). During the acute phase in patients with HAEC, occludin downregulation to 40-60% of baseline levels confers cytoprotection by inhibiting caspase-3-mediated apoptosis (59). However, sustained occludin deficiency induces barrier collapse via TJ endocytosis (50,52). Notably, the spatiotemporal dynamics of this duality in the effects of occludin downregulation still require investigation using conditional gene-editing organoid models. Future research should focus on: i) Subcellular dynamic imaging of the occludin/caveolin-1 interaction to reveal real-time regulation in lipid raft microdomains; ii) development of phosphorylation site-specific antibodies for precise

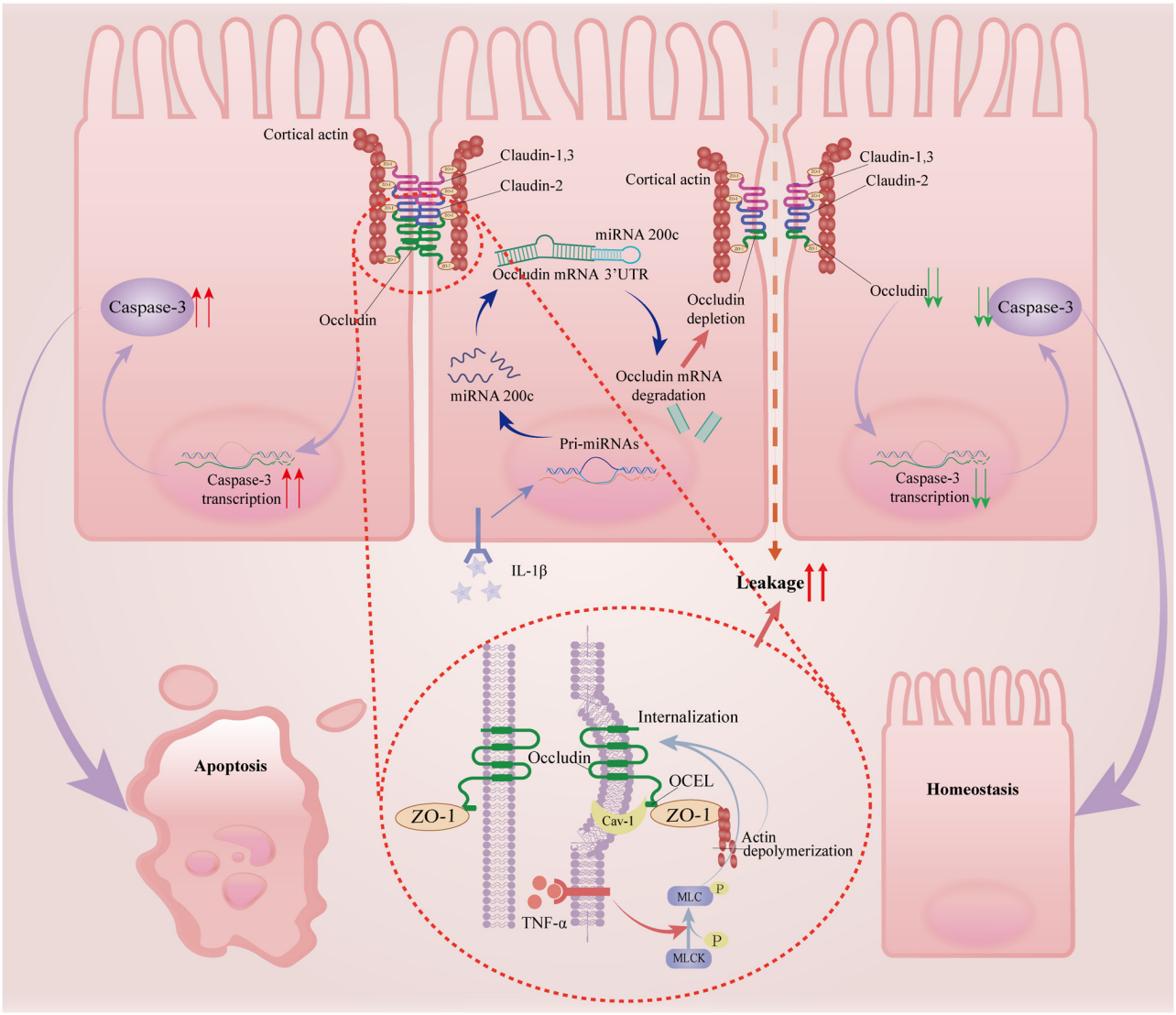

Figure 2. Potential roles of occludin in HAEC. IL-1β upregulates the expression of miRNAs that bind to occludin mRNA, inducing its degradation or translational suppression, thereby reducing occludin expression. This alteration increases intestinal epithelial permeability, disrupts the intestinal barrier function and promotes the development of HAECs. Inflammatory signals such as TNF-α activate the MLCK pathway, leading to MLC phosphorylation and mediating the internalization of occludin through the OCEL domain, while simultaneously triggering a caveolin-1-dependent endocytic cascade. At the same time, actin depolymerization triggers the internalization of cholesterol-enriched membranes, further promoting the internalization of occludin and increasing the permeability of paracellular pathways. On the other hand, occludin enhances the transcription of caspase-3, promoting cell apoptosis. Under inflammatory conditions, the downregulation of occludin may confer an anti-apoptotic ability to cells, thus helping to maintain mucosal homeostasis and protecting the intestinal barrier. HAEC, Hirschsprung-associated enterocolitis; ZO-1, zonula occludens protein 1; miRNA, microRNA; Pri-miRNA, primary miRNA; Cav-1, caveolin-1; MLCK, myosin light chain kinase; P, phosphate group; OCEL, occludin/ELL; MLC, myosin light chain; UTR, untranslated region.

detection of pathological states of occludin; and iii) screening of novel therapeutic molecules targeting the OCEL domain to restore barrier function without interfering with structural integrity.

4. ZO-1

Molecular structure and functional scaffold. As a representative member of the membrane-associated guanylate kinase protein family, ZO-1 mediates multidimensional regulation of TJs through its modular structure: i) Three PDZ domains guide the topological localization of claudin/occludin; ii) the SRC homology 3 (SH3) domain recruits signaling kinases to regulate downstream pathways; and iii) the actin-binding region (ABR) at the carboxyl terminus forms the physical-functional coupling interface between TJs and the cytoskeleton (60,61). Notably, in ZO-1 knockout models, although TJ ultrastructure can spontaneously assemble (62), its maturation is significantly delayed and accompanied by increased macromolecular transmembrane leakage (63), suggesting that ZO-1 is not a rigid scaffold for TJ assembly. Rather, ZO-1 dynamically regulates the liquid-liquid phase transition of protein complexes through a phase separation mechanism. ZO-1 phase separation, mediated through PDZ-SH3 domain-driven biomolecular condensate formation, orchestrates rapid TJ assembly by concentrating scaffolding proteins (Fig. 3) (60). Notably, pro-inflammatory cytokines such as TNF-α disrupt this process by inducing hyperphosphorylation of the intrinsically disordered regions of ZO-1, thereby dissolving condensates and impairing barrier repair (64). In intestinal epithelia, TNF-α

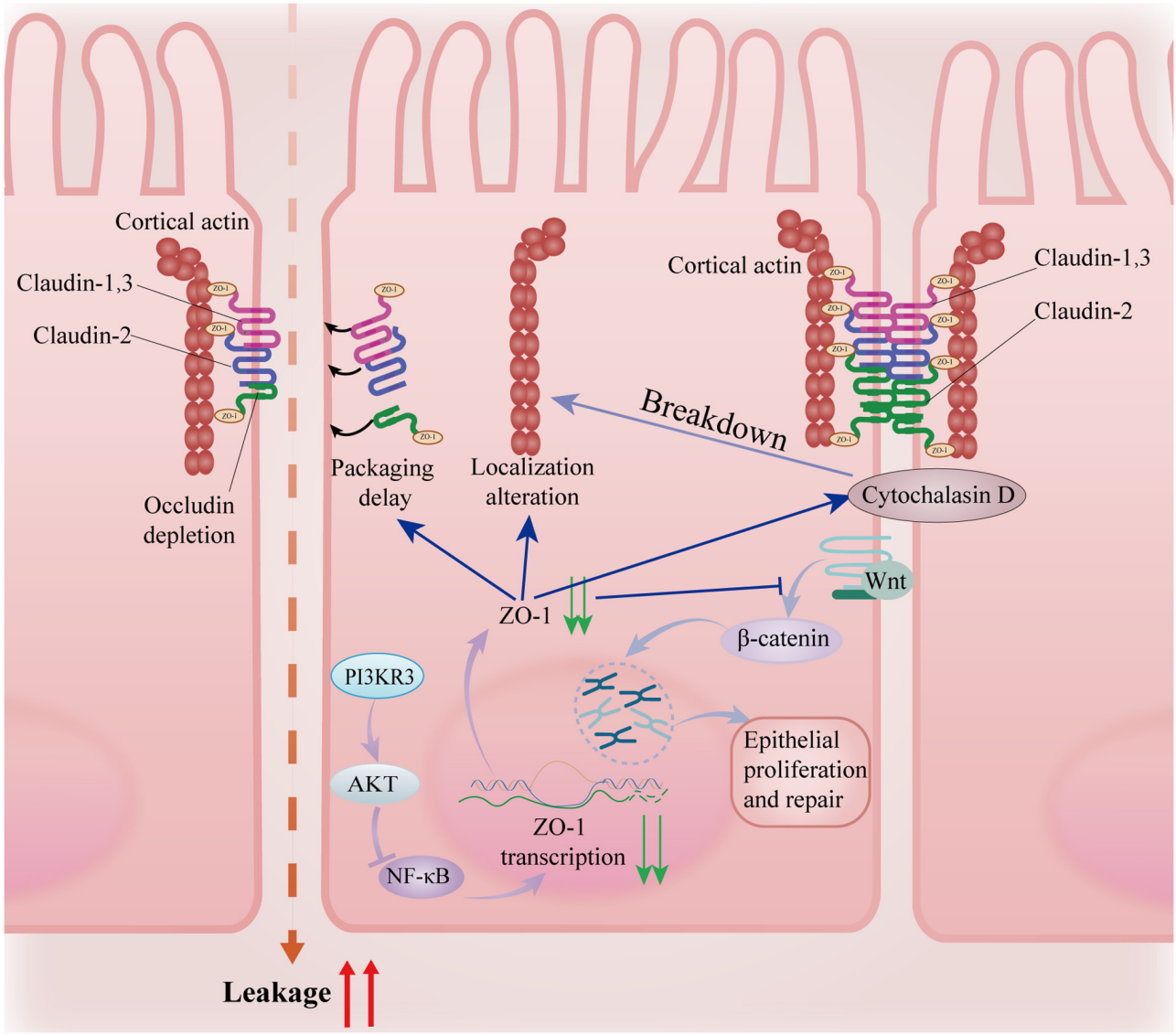

Figure 3. Potential roles of ZO-1 in HAEC. Upregulation of PI3K regulatory subunit 3 can induce NF-κB activation and reduce ZO-1 expression. A deficiency in ZO-1 leads to delayed recruitment and assembly of claudins and occludin at TJs and affects actin distribution, thereby impairing TJ barrier function. Additionally, downregulation of ZO-1 weakens Wnt/β-catenin signaling, leading to excessive proliferation of epithelial cells and disrupting mucosal repair. Furthermore, ZO-1 downregulation increases the sensitivity of intestinal epithelial cells to cytochalasin D, which disrupts the actin cytoskeleton, resulting in TJ barrier dysfunction, increased epithelial permeability and promotion of HAEC development. HAEC, Hirschsprung-associated enterocolitis; ZO-1, zonula occludens protein 1; TJ, tight junction; PI3KR3, PI3K regulatory subunit 3.

reduces ZO-1 expression while increasing phosphorylation of its intrinsically disordered regions, directly impairing barrier function through delayed TJ assembly (65). This multifaceted structural-functional characteristic positions ZO-1 as both a molecular adapter and a signaling hub, with conformational changes potentially regulating barrier plasticity through allosteric effects.

Dynamic regulatory mechanisms. ZO-1 regulates TJ plasticity through two synergistic axes: i) ZO-1 directly interacts with claudin proteins and guides the localization of their polymerization sites (66), coordinating the assembly of transmembrane proteins; and ii) ZO-1 couples TJs to the cytoskeletal network via its ABR, with actin depolymerization having been shown to significantly increase paracellular permeability (67). It is worth noting that although ZO-1 deletion does not alter the total actin content of the cell, it disrupts its spatial organization (68), highlighting the role of ZO-1 in the topological regulation of the cytoskeleton rather than providing only mechanical anchorage. This dual regulatory mechanism may provide insights into how ZO-1 coordinates acute barrier remodeling and chronic fibrosis during mucosal repair.

Mucosal repair mechanism and pathological dysregulation. Upon injury, ZO-1 coordinates epithelial repair through the Wnt/β-catenin signaling pathway (Fig. 3) (69). ZO-1 knockdown reduces β-catenin nuclear translocation and impairs wound healing (69). Clinical cohort studies have shown a strong positive correlation between ZO-1 expression in intestinal epithelium and endoscopic healing scores in patients with IBD (69), while ZO-1 mRNA levels in exosomes from fecal samples of neonates with NEC can predict the risk of intestinal

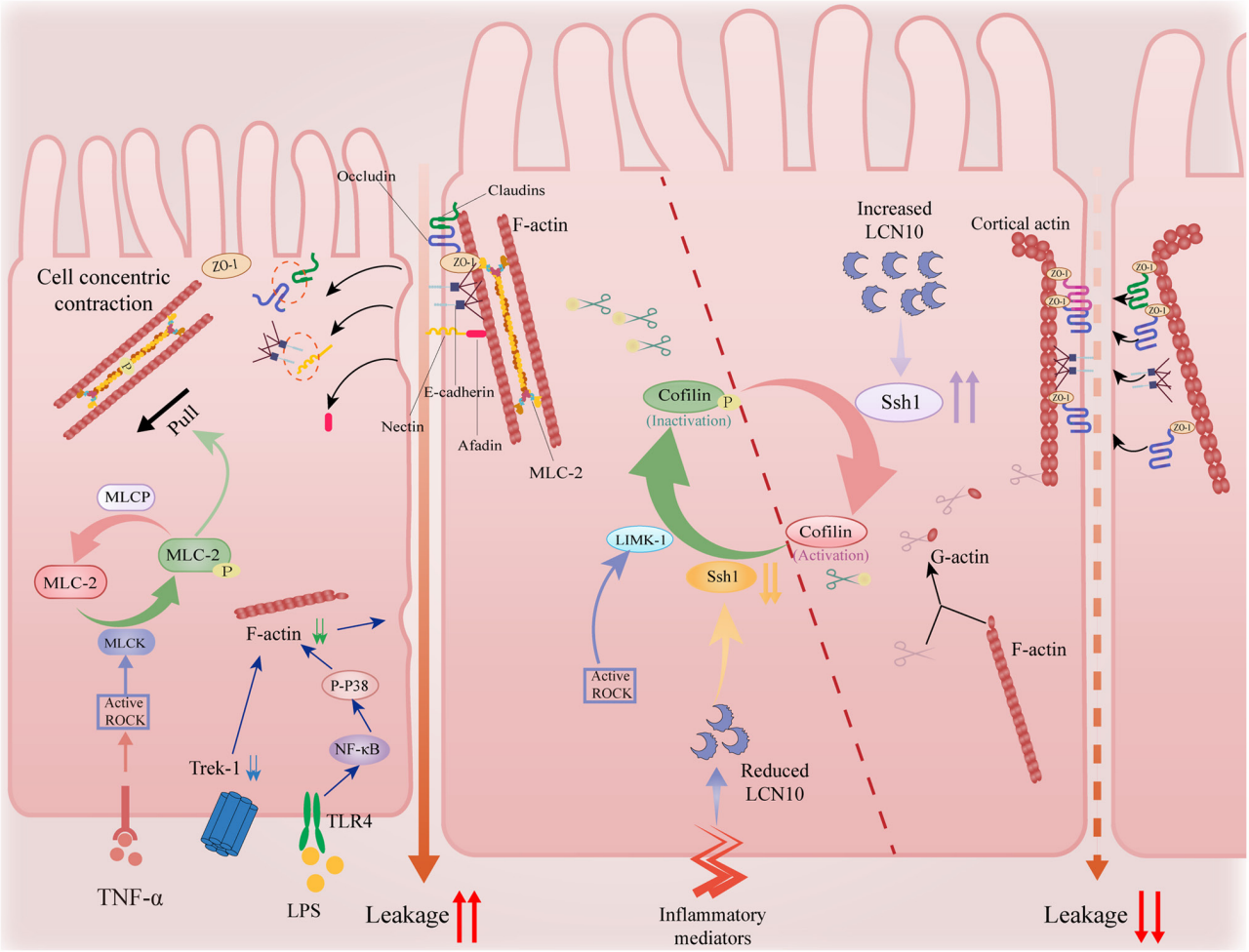

Figure 4. Potential roles of actin in HAEC. Inflammatory mediators released by macrophages cause a significant decrease in LCN10 expression, inactivation of Ssh1 and increased phosphorylation of cofilin. These changes result in suppressed F-actin depolymerization, a heightened F-actin/G-actin ratio and subsequent stress fiber formation. The consequent maldistribution of tight junction proteins and loss of barrier integrity increase epithelial permeability, thereby exacerbating the progression of HAEC. Increased intracellular LCN10 can induce the dephosphorylation of Ssh1/cofilin, promoting the depolymerization of F-actin into G-actin, which helps repair TJ disassembly, reduces paracellular permeability and protects intestinal barrier function. Additionally, TNF-α activates the RhoA/ROCK signaling pathway, which subsequently phosphorylates MLCK. This cascade induces phosphorylation of MLC-2, triggering actin contraction, increased paracellular permeability and intestinal barrier dysfunction. Furthermore, ROCK activates LIMK to phosphorylate cofilin, thereby stabilizing F-actin polymers and amplifying cytoskeletal remodeling. LPS stimulates NF-κB via TLR4 and significantly reduces F-actin density through NF-κB/phosphorylated p38 signaling. Cells with Trek-1 gene deficiency show lower F-actin levels, leading to the opening of paracellular channels, disruption of intestinal barrier function and promotion of HAEC development. HAEC, Hirschsprung-associated enterocolitis; ZO-1, zonula occludens protein 1; TJ, tight junction; G-actin, globular-actin; F-actin, filamentous actin; MLCK, myosin light chain kinase; MLC-2, myosin light chain-2; LCN10, lipocalin-10; Ssh1, slingshot homologue 1; Trek-1, Twik-related K^+ channel-1; TLR4, Toll-like receptor 4; ROCK, Rho-associated protein kinase; LIMK, LIM kinase; MLCP, myosin light chain phosphatase; P, phosphate group; LPS, lipopolysaccharide.

perforation (57). These findings collectively suggest that ZO-1 not only mediates barrier integrity but also acts as an important node linking homeostasis maintenance and regenerative repair through mechanical-chemical signaling. Its functional depletion may trigger a cycle of barrier defect-induced chronic inflammation.

Potential role of ZO-1 in HAEC. Although ZO-1 plays a central role in barrier regulation, its pathological mechanism in congenital megacolon-associated fatal complications, such as HAEC, remains yet to be fully elucidated. Given that HAEC is characterized by the disruption of TJ structures (17), targeting the regulation of ZO-1 interactions offers a promising avenue for therapeutic innovation. Future research may explore the spatiotemporal analysis of ZO-1 dynamics in HAEC models to identify important nodes of dysfunction, for example ABR-actin uncoupling and other pathological processes. Furthermore, peptides derived from the ABR could be designed to selectively block pathological cytoskeletal remodeling while preserving barrier integrity. The systematic integration of these research strategies will drive translational medical progress from mechanism elucidation to precise intervention in HAEC.

5. Actin

Molecular regulation of actin dynamics. The plasticity of the intestinal epithelial cell barrier relies on the spatiotemporal reorganization of the actin cytoskeleton. The steady-state conversion between globular-actin monomers and F-actin

polymers is finely regulated by the phosphorylation cycle of cofilin. LIM kinase (LIMK) mediates cofilin phosphorylation, thereby inhibiting its activity, while the phosphatase slingshot homologue 1 (Ssh1) activates cofilin through dephosphorylation, promoting the disassembly of F-actin (Fig. 4) (70,71). Imbalance in this system can directly disrupt the membrane localization of TJ proteins, such as ZO-1 and occludin, leading to an increase in paracellular permeability (23,72).

Mechanical signal transduction via ZO-1/F-actin interaction. ZO-1 forms a mechanical coupling interface with F-actin through its carboxy-terminal ABR. Loss of ZO-1 function results in the disruption of actin filament integrity (68). MLCK and ROCK mediate actomyosin contraction through phosphorylation of myosin light chains, which, in turn, dissociates the ZO-1 ABR from F-actin, ultimately triggering the opening of the paracellular pathway (73-75). Notably, MLCK inhibitors completely reverse the ZO-1 internalization phenotype, while ROCK inhibitors partially restore barrier function, indicating the signaling pathway specificity of the ZO-1/F-actin interaction (76,77). This mechanical transduction property positions ZO-1 as a molecular sensor linking the mechanical microenvironment with barrier plasticity.

Cytoskeletal remodeling network in inflammatory environments. Pro-inflammatory factors exacerbate barrier damage via multifaceted regulation of actin dynamics: i) IFN-γ enhances actomyosin contractility via a ROCK-dependent pathway, leading to the internalization of ZO-1 and occluding (78); ii) a dynamic interaction exists between Twik-related K^+ channel-1 (Trek-1) and the actin cytoskeleton. In colonic epithelia, Trek-1 stabilizes cortical actin via direct binding to F-actin. Trek-1 deficiency reduces F-actin density, increasing paracellular permeability (79). Hypoganglionic segments in HSCR show lower Trek-1 expression compared with normoganglionic bowels. This deficit impairs mechanotransduction in the neurogenic microenvironment, exacerbating barrier dysfunction during HAEC flares (Fig. 4) (80). Similarly, activation of the Toll-like receptor 4/phosphorylated p38/NF-κB signaling pathway also results in reduced F-actin expression (81), influencing the opening of intercellular gaps (Fig. 4); and (iii) lipopolysaccharides (LPS) inhibit EGFR phosphorylation, thereby hindering its activity in protective actin reorganization (82). Collectively, these pathways form a positive feedback loop of inflammatory signals/cytoskeletal remodeling/barrier breakdown, providing a mechanistic explanation for the chronic progression of IBD.

Translational insights of the LCN10/Ssh1/cofilin axis. The lipid carrier protein LCN10, a secreted protein expressed in macrophages, endothelial and epithelial cells (64), serves as an important regulator of cytoskeletal dynamics. Mechanistically, LCN10 activates Ssh1 phosphatase to dephosphorylate cofilin, thereby enhancing F-actin depolymerization, a process important for TJ protein redistribution in barrier dysfunction (42,83). Notably, this LCN10/Ssh1/cofilin pathway-dependent regulation is conserved across vascular and intestinal barriers, where LCN10 deficiency exacerbates inflammation-induced permeability (42). Pathologically, pro-inflammatory stimuli, such as LPS and IFN-γ, suppress LCN10 expression by >80% in intestinal macrophages (41), which may drive HAEC progression by disrupting actin-mediated TJ stability.

Furthermore, in cohorts of pediatric patients with HAEC (n=75), stenotic bowel segments exhibit 73% lower phosphorylated cofilin levels vs. controls (84,85). Mechanistically, LCN10 activates Ssh1 via high-affinity binding to low-density lipoprotein receptor-related protein 2, restoring cofilin-mediated actin dynamics to reduce barrier leakage (42). These findings nominate the LCN10/Ssh1/cofilin axis as a theragnostic target for HAEC. Based on these findings, small molecule agonists targeting LCN10 may overcome the current lack of cytoskeletal targets in HAEC therapies. Furthermore, we hypothesize that combinatory strategies involving ZO-1 ABR stabilizers could produce synergistic barrier repair effects.

6. Conclusion

The present study elucidates the molecular pathogenesis of HAEC, revealing that intestinal barrier dysfunction stems from TJ protein dysregulation, including claudin-2/4 imbalance, occludin endocytosis via the MLCK/NF-κB pathway and ZO-1-cytoskeleton uncoupling, coupled with inflammatory mediator-driven actin remodeling through the cofilin phosphorylation cycle. The identification of the LCN10/Ssh1/cofilin axis and TJ-cytoskeleton interactions provides mechanistic insights into HAEC pathogenesis and potential therapeutic targets. The scarcity of targeted research on TJ and cytoskeletal proteins in HAEC underscores the translational significance of the present study. Future studies should investigate subcellular TJ protein dynamics, develop small-molecule modulators of the LCN10/Ssh1 pathway, validate their pharmacokinetic and toxicological profiles and explore combination therapies targeting both barrier repair and inflammation, ultimately translating these findings into clinical strategies for HAEC prevention and treatment.

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Availability of data and materials

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Authors' contributions

ST, YW and LL contributed to the conception and design of the study, and provided administrative support. LZ, ShaC, YZ, DY, KL, YL and ShuC participated in data analysis and visualization, including the creation and interpretation of graphical figures. SL and CW were major contributors in drafting and revising the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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