Advances in Research on Myosin Light Chain Kinase-Mediated Alterations in Intestinal Epithelial Barrier Function (Postprint)

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Abstract

Alterations in intestinal mucosal permeability are intimately associated with the regulation of myosin light chain kinase (MLCK). The cytoskeletal contraction elicited by MLCK-mediated phosphorylation of myosin light chain (P-MLC) represents an essential factor in the disruption of intestinal mucosal epithelial barrier function. Diverse signaling molecules can activate MLCK through distinct signaling pathways, thereby inducing dysfunction of the intestinal mucosal epithelial barrier. This review systematically delineates the structure and biological functions of MLCK, as well as its regulatory effects and underlying mechanisms on the intestinal mucosal epithelial barrier across various signaling pathways, aiming to provide conceptual frameworks and theoretical support for future development of novel therapeutic strategies against intestinal mucosal epithelial barrier injury.

Full Text

Research Progress on Myosin Light Chain Kinase-Mediated Regulation of Intestinal Mucosal Epithelial Barrier Function

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Abstract: Alterations in intestinal mucosal permeability are closely associated with the regulation of myosin light chain kinase (MLCK). The contraction of the cytoskeleton induced by MLCK-mediated phosphorylated myosin light chain (P-MLC) represents an essential factor in the disruption of intestinal mucosal

epithelial barrier function. Multiple signaling molecules can activate MLCK through distinct signaling pathways, thereby causing intestinal mucosal epithelial barrier dysfunction. This review elaborates on the structure and biological functions of MLCK, as well as its regulatory roles and mechanisms in intestinal mucosal epithelial barrier function across different signaling pathways, aiming to provide insights and theoretical support for developing novel technical measures to prevent and treat intestinal mucosal epithelial barrier damage.

Keywords: myosin light chain kinase; myosin light chain; intestinal mucosal epithelium; permeability; mechanism

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The intestine serves as the primary site for nutrient digestion and absorption in animals, while the mucosal layer functions as a critical barrier that effectively prevents the diffusion of intestinal microorganisms and their toxins into other tissues, organs, and the circulatory system [1], maintaining dynamic stability during the invasion-anti-invasion process [2]. The integrity of the intestinal mucosal epithelial barrier plays a vital role in maintaining the health of the digestive system and the entire organism. When this barrier is compromised or dysfunctional, it promotes the development of intestinal infectious diseases and can lead to systemic inflammatory responses or multiple organ failure in severe cases [3-4]. Myosin light chain kinase (MLCK) is the most important calmodulin-dependent kinase involved in intestinal mucosal epithelial barrier function changes. With in-depth research on its structure and function, the role of MLCK in mediating intestinal mucosal epithelial permeability changes has attracted widespread attention from scholars and has become a hotspot in molecular and cell biology research. Therefore, understanding the regulatory mechanisms of MLCK-mediated intestinal mucosal epithelial barrier function changes holds significant importance for animal nutrition research.

1.1 Basic Structure of MLCK

MLCK was the first serine/threonine-specific protein kinase discovered to be dependent on calmodulin (CaM), dynamically regulating actomyosin recombination and cell contraction in muscle cells of eukaryotes and in non-muscle cells of mammals [5]. In mammals, MLCK is primarily encoded by two genes, *mylk1* and *mylk2* [6], and can be divided into skeletal muscle MLCK (skMLCK) and smooth muscle MLCK (smMLCK), which are located on different chromosomes. skMLCK is exclusively expressed in skeletal muscle tissue and encodes a catalytic domain and a regulatory region comprising an autoinhibitory zone and Ca^{2} /CaM binding sequences [7]. Due to different promoters, smMLCK expresses three transcript isoforms in a cell-specific manner. The 130 kDa isoform, termed short-chain MLCK (S-MLCK), is expressed in most tissues and plays an important regulatory role in maintaining basic gastrointestinal tone, gastric emptying, and small intestinal propulsion [8]. The 220 kDa isoform, known as long-chain MLCK (L-MLCK), is mainly distributed in embryonic tissues, cul-

tured cells, epithelial cells, and non-muscle cells [9]. Research has shown that phosphorylation of myosin light chain (MLC) in intestinal epithelial cells is primarily caused by L-MLCK expression [10]. The third transcript isoform of the smMLCK gene encodes a C-terminal immunoglobulin T (IgT)-like structure.

Feng et al. [11] identified the conserved domains of mammalian smMLCK, as illustrated in [Figure 1: see original paper]. The N-terminal actin-binding site of smMLCK consists of three DFRxxL motifs that bind purified F-actin, anchoring the enzyme within the contractile apparatus [12-13]. Two immunoglobulin domains, Ig1 and Ig2, also bind actin [14], while the IgT domain binds C-terminal smooth muscle myosin (SMM), a structural protein crucial for maintaining myosin filament stability in smooth muscle cells [15]. The central kinase domain serves as the catalytic site for MLCK, adenosine triphosphate (ATP), and regulatory myosin II light chain (RLC) binding, transferring phosphate from ATP to substrates [16].

Figure 1 Domain structure of smMLCK in mammals [11]

Additionally, MLCK-related kinases have been identified in invertebrates. In *Drosophila*, a gene with complex promoters produces multiple transcripts sharing identical termini [17]. Smaller transcripts (3.2–5.2 kb) encode proteins similar in size to mammalian MLCK, while larger transcripts (13–25 kb) encode proteins resembling mammalian titins. The largest transcript (25 kb) encodes a 926 kDa stretchin-MLCK. In *Caenorhabditis elegans* and *Aplysia*, the expressed MLCK-related kinase is twitchin, which contains an autoinhibitory region attached to the catalytic domain but lacks Ca^2 /CaM binding sequences, and is activated by the Ca^2 -binding protein S100A12 [18]. The MLCK expressed in *Dictyostelium discoideum* contains only a catalytic domain and a regulatory region that is activated by phosphorylation, representing the simplest structural form [19].

1.2 Biological Functions of MLCK

MLCK is a key regulator of cell contraction, primarily functioning to mediate MLC phosphorylation [20-21]. Ca^2 /CaM represents the most important regulator of MLCK activity. Upon stimulation by various external signals, intracellular free Ca^2 concentration increases. Ca^2 first binds to CaM to form the Ca^2 /CaM complex, which then binds to MLCK to relieve its autoinhibitory sequence, forming activated p-MLCK [7,11]. Activated p-MLCK elevates phosphorylation levels at threonine 18 (Thr18) and serine 19 (Ser19) residues on MLC, altering MLC spatial conformation [22]. Phosphorylated MLC activates the ATPase in the myosin heavy chain head, generating energy for actin-myosin interaction that mediates actin contraction. Ca^2 /CaM can also bind to DFRxxL motifs, weakening actin binding [23], causing cytoskeletal actin microfilament sliding, inducing centripetal cell contraction, disrupting intercellular tight junctions (TJ), and directly catalyzing the transition of MLC from non-phosphorylated to phosphorylated forms, ultimately increasing cellular mucosal permeability [24].

Furthermore, studies by Kamm et al. [7] and Simard et al. [25] have demonstrated that MLCK not only regulates cell contraction but also modulates cell migration, motility, and apoptosis.

2 Signal Transduction Mechanisms of MLCK-Mediated Intestinal Mucosal Epithelial Barrier Function Changes

The mechanical barrier, as a crucial component of the intestinal mucosal barrier, is primarily composed of intestinal epithelial cells and intercellular junctions. Cell coordination and functional integration depend on cell adhesion and junctions. Cell junctions are mainly classified into tight junctions (TJ), adhesion junctions (AJ), gap junctions (GJ), and desmosome junctions (DJ) [26]. Among these, TJ represent the most important connection mode between intestinal epithelial cells, regulating the transepithelial transport of water, solutes, and small molecules, and serving as the key determinant of intercellular permeability [27]. TJ play a pivotal role in maintaining intestinal mucosal epithelial barrier function. Blair et al. [28] found that MLCK can increase intestinal mucosal permeability by regulating protein expression of Claudin, Occludin, and ZO proteins, indicating that MLCK plays an important role in the dynamic regulation of TJ permeability [29]. Research has shown that altered epithelial cell contractility represents a common pathway for increased intestinal mucosal permeability induced by various factors, primarily influenced by actin and myosin in the cytoskeleton. Myosin mainly regulates cytoskeletal structure and participates in various cellular physiological activities, which are primarily achieved through MLC phosphorylation and dephosphorylation. MLC phosphorylation constitutes the molecular basis for increased biological barrier permeability [30-31] and represents the key to intestinal epithelial TJ barrier dysfunction. Various cytokines, inflammatory mediators, and neurohumoral factors can increase mucosal permeability through MLC phosphorylation [32-33]. Therefore, in MLCK-mediated increases in intestinal mucosal epithelial cell permeability, effector molecule MLC phosphorylation is the critical link, while MLCK activation can be regulated through the following pathways.

2.1 Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway

MAPK belongs to the serine/threonine protein kinase family and was first purified from 3T3-L1 adipoblasts by Sturgill et al. [34]. Research has confirmed that MAPK exists in most cells of all organisms and represents one of the important signal transduction systems in eukaryotic cells, transmitting extracellular signals to the cell interior and nucleus. MAPK influences cellular biological functions by affecting gene transcription and regulation [35]. The MAPK signal transduction pathway is highly conserved in biological evolution. Currently, the MAPK signaling system mainly includes extracellular signal-regulated kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase (JNK). ERK plays an important role in maintaining cell morphology and cytoskeletal construction. Studies have shown that ERK1/2 activation can promote downstream transcription activa-

tor ETS-like protein 1 (Elk-1) activation and nuclear translocation, which binds to cis-acting sites within the minimal promoter region (-310 to -296), triggering MLCK gene activation and MLC phosphorylation, leading to impaired intestinal epithelial TJ and increased mucosal permeability [36]. Additional research indicates that homocysteine affects intestinal mucosal permeability in colitis rats by promoting MEK-ERK-MLCK protein phosphorylation, thereby aggravating intestinal inflammation [37]. Al-Sadi et al. [36] found that interleukin-1 (IL-1)-induced increases in intestinal epithelial cell TJ permeability are mediated through ERK1/2 signal transduction pathway regulation of MLCK gene expression, and that MLC phosphorylation can be effectively inhibited by ERK1/2 knockout or ERK1/2 inhibitors. Therefore, ERK plays an important role in MLCK-induced increases in intestinal mucosal epithelial cell permeability. In mouse intestinal barrier function injury caused by burns, the p38 MAPK signaling pathway can activate MLCK, leading to altered intestinal mucosal tissue morphology and increased TJ permeability between epithelial cells. Injection of p38 MAPK inhibitors reduces p38 MAPK phosphorylation levels and MLCK gene expression. Thus, the MAPK signaling pathway occupies an important position in MLCK-mediated intestinal mucosal epithelial barrier dysfunction and cell permeability changes.

2.2 MLCK-Mediated MLC Phosphorylation Signaling Pathway

Intestinal mucosal permeability changes are closely related to MLCK regulation, and MLC phosphorylation is the key to intestinal mucosal epithelial TJ barrier dysfunction. Moriez et al. [38] found that after lipopolysaccharide injection, rat epithelial cell TJ expanded, MLCK was activated, and MLC phosphorylation increased, causing cell contraction and intercellular gap formation that ultimately affected colonic mucosal permeability. Injection of the MLCK-specific inhibitor ML-7 significantly reduced MLCK activity and its induced barrier dysfunction. Studies have reported that in monolayer epithelial cell models treated with inflammatory cytokines tumor necrosis factor- (TNF-) and interferon- (IFN-), MLCK expression levels were upregulated, MLC phosphorylation was significantly elevated, and epithelial cell barrier function was damaged, while these effects could be ameliorated by MLCK inhibitors [39]. These results indicate that the MLCK-mediated MLC phosphorylation signaling pathway plays an important role in the mechanism of intestinal epithelial barrier damage induced by endotoxins or different inflammatory factors.

Chen Chuanli [40] demonstrated that increased intestinal mucosal permeability caused by severe early burns and hypoxia in mice was accompanied by increased MLCK protein expression and MLC phosphorylation levels, which were suppressed by injection of the ML-9 inhibitor. Additionally, the MLCK-mediated MLC phosphorylation signaling pathway is involved in heat stress-induced intestinal mucosal epithelial barrier damage, and injection of the ML-7 specific inhibitor can prevent MLC phosphorylation and increased intestinal mucosal epithelial permeability. Besides MLCK regulation, MLC phosphorylation

is also negatively regulated by myosin light chain phosphatase (MLCP) [41]. Rho kinase (ROCK) can interact with MLCP subunits, causing MLCP inactivation and thereby preventing MLCP-mediated MLC dephosphorylation, which increases intracellular MLC phosphorylation levels [42]. Activation of intestinal mucosal ROCK and increased MLC phosphorylation levels after severe burns constitute one of the molecular mechanisms leading to increased intestinal mucosal permeability and barrier dysfunction in rats. Therefore, ROCK activation represents another cause of MLC phosphorylation.

2.3 Protein Kinase C (PKC)

PKC, discovered in the 1970s, is a class of Ca^2-activated, phospholipid-dependent protein kinases widely distributed in mammalian tissues, organs, and cells. Through catalyzing protein phosphorylation, PKC plays important regulatory roles in animal cell growth, differentiation, metabolism, information transfer, and signal transduction [43]. PKC has a molecular mass of 70–90 kDa and consists of an N-terminal regulatory region and a C-terminal catalytic region. Once activated, this protein kinase translocates to the cell membrane to phosphorylate protein substrates and trigger numerous intracellular responses, though the activated PKC isoforms and main pathways differ among cell types [44]. As a protein kinase, PKC can directly act on serine/threonine residues of MLC to cause MLC phosphorylation, or indirectly activate MLCK to induce cytoskeletal protein MLC phosphorylation and subsequent reorganization of structural proteins [45]. Studies have shown that after PKC activation in intestinal mucosal epithelial cells, the phosphorylation status and enzymatic activity of MLCK change, leading to altered MLC phosphorylation status that affects the contraction of the perijunctional actomyosin ring, ultimately increasing intestinal mucosal epithelial permeability [46]. Therefore, PKC can mediate changes in intestinal mucosal epithelial barrier function through MLCK phosphorylation.

2.4 Ca^2 Concentration

Ca^2 plays an important role in maintaining normal physiological functions of intestinal mucosal epithelium, and changes in intracellular free Ca^2 concentration regulate cellular energy metabolism, protein phosphorylation and dephosphorylation modifications, gene expression, and regulation [47]. Ca^2 is the most fundamental mediator regulating MLCK activity, and its binding to CaM to activate MLCK represents a crucial factor in determining MLC phosphorylation and inducing cell contraction [23,48]. Studies have shown that when extracellular Ca^2 concentration decreases, intracellular MLCK activity is activated, actin and myosin undergo centripetal contraction, intercellular TJ are disrupted, and intestinal mucosal epithelial barrier function is damaged, leading to increased permeability [49]. Ma et al. [50] found that Ca^2-induced changes in intestinal mucosal epithelial TJ barrier function are related to MLCK activation, and that MLCK inhibitor ML-7 can prevent MLCK activation and increased intestinal

mucosal epithelial cell permeability, demonstrating that Ca^2 increases intestinal mucosal epithelial permeability through MLCK activation. Additionally, Ca^2 channels are specific membrane proteins related to Ca^2 transport, and their activation is crucial for regulating intracellular and extracellular Ca^2 concentrations, playing an important role in the process of MLCK-mediated increases in intestinal mucosal epithelial permeability.

3 Summary

Multiple signaling molecules can activate MLCK through different signal transduction pathways, leading to intestinal mucosal epithelial barrier dysfunction. Among these, MLCK-mediated MLC phosphorylation is the key link in MLCK-mediated increases in intestinal permeability and serves as the central hub for various intracellular signaling pathways. Increased MLCK activity and protein expression levels can both cause elevated MLC phosphorylation, altered intercellular TJ, cell contraction, and enlarged intercellular gaps, thereby affecting intestinal mucosal epithelial barrier function and increasing permeability. In recent years, significant progress has been made in understanding the mechanisms of MLCK-mediated intestinal mucosal epithelial barrier damage. However, research in this area has primarily focused on humans and monogastric animals, with limited studies in ruminants. Therefore, it is necessary to further explore the effects of MLCK on ruminant intestinal mucosal epithelial barrier function and its molecular regulatory mechanisms. Meanwhile, with the advent of the big data era, bioinformatics has been widely applied in research on metabolic diseases in animal nutrition, which provides guidance for better identification of key signaling pathways and related upstream and downstream functional genes involved in MLCK-mediated changes in ruminant intestinal mucosal permeability, and offers theoretical support for exploring novel technical measures to prevent and treat intestinal mucosal epithelial barrier damage.

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