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REVIEW

AMPK in regulation of apical junctions and barrier function of intestinal epithelium

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ABSTRACT

Gut epithelium covers the inner layer of the gastrointestinal tract and provides a physical barrier to separate the host from its external environment, and its barrier function is critical for maintaining host health. AMP-activated protein kinase (AMPK) as a master regulator of energy metabolism plays a critical role in epithelial barrier function. AMPK activation promotes epithelial differentiation and facilitates cell polarity establishment, both of which strengthen epithelial barrier. In addition, AMPK promotes the assembly of tight junctions and adherens junctions by direct phosphorylation of proteins composing apical junctions, junctional anchors, and cytoskeletons. Pharmacological and nutraceutical compounds, as well as physiological states triggering AMPK activation strengthen epithelial barrier function. This review summarized recent progress in delineating the regulatory roles of AMPK in apical junction formation and barrier function of intestinal epithelium.

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adherens junction; AMPK; barrier function; epithelial differentiation; tight junction

Introduction

Epithelium forms a physical barrier to perform both fence and gate functions, which protect against the penetration of noxious substances and allow the passage of nutrients, ions and small solutes.[1,2] Intestinal epithelium constitutes a single layer of epithelial cells that are constantly renewed.[3] Epithelial cells are tightly linked through apical junctions, including tight junctions (TJs) and adherens junctions (AJs).[4] The functionality of epithelial barrier is orchestrated by delicate balances among epithelial proliferation, differentiation and apoptosis, as well as apical junction formation and assembly.[1] The disturbance of junctional organization leads to increased permeability of the intestinal epithelium, or "leaky gut".[5–9] Subsequently, dysfunctional barrier function could cause various diseases, such as inflammatory bowel diseases (IBD),[10,11] hypercalciuria and hypomagnesemia kidney diseases,[12] and autoimmune diseases.[13] In addition, endotoxemia due to the leaky gut barrier is associated with metabolic diseases,[14] Parkinson's,[15] Alzheimer's,[16] and multiple sclerosis.[17]

AMP-activated protein kinase (AMPK), a master regulator of energy metabolism,[18,19] is increasingly known for its vital role in regulating myogenesis,[20] adipogenesis[21] and cardiac differentiation.[22] We recently reported that AMPK plays an important role in regulating gut epithelial differentiation and barrier function.[8,23] Pharmacological, nutraceutical and physiological activation of AMPK strengthens epithelial apical junctions and protects epithelial barrier against environmental stresses,[24–28] while AMPK inhibition due to metabolic disorders is concurrent with impaired epithelial barrier function.[18,29] This review summarizes the recent literature on favorable effects of AMPK on epithelial apical junction assembly and intestinal barrier function.

Epithelial apical junction overview

Epithelial cells are joined by a series of intercellular junctions and polarized into the apical and the basolateral domains.[30,31] The apical domain of epithelial cells is linked with adjacent epithelial cells through TJs and AJs, which are also referred to as apical junctions (Fig. 1). The assembly of apical junctions is indispensable for the formation and maintenance

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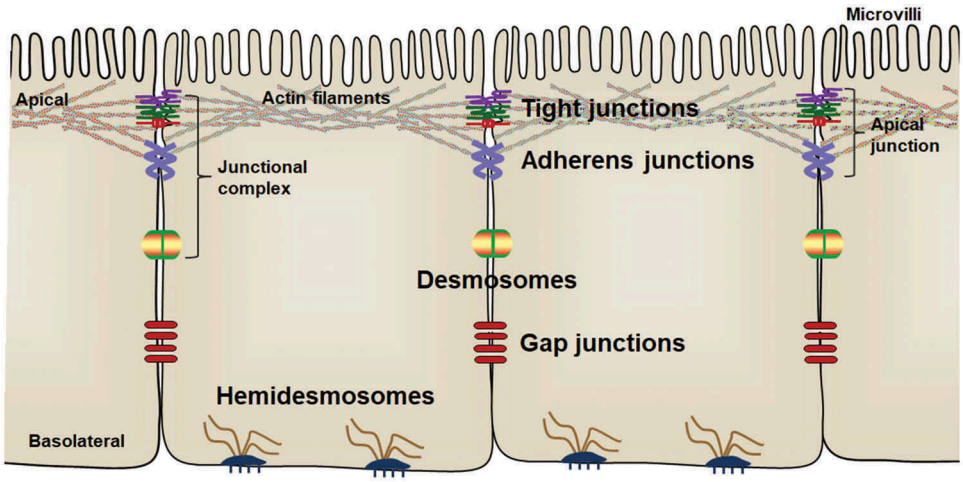


Figure 1. Arrangement of intestinal epithelial cells and intercellular junctions between epithelial cells. The apical junctions are composed of tight junctions and adherens junctions.

of epithelial barrier integrity.[1,32] Desmosomes are intercellular junctions located below AJs on the lateral membrane and link to intermediate filaments to stabilize the epithelial layer and provide mechanical strength to tissues.[33,34] On the basolateral membrane, hemidesmosomes connect to intermediate filament and facilitate epithelial cell adhesion to extracellular matrix in the basal lamina.[35] In addition, epithelial cells communicate with surrounding cells through gap junctions (Fig. 1) that are composed of connexins and assembled into hexameric pore-forming channels.[36]

Epithelial tight junctions

Tight junctions are localized at the most apical constituent of the intestinal epithelium, regulate paracellular permeability and contribute to epithelial cell polarity.[37] Primary constituents of TJs are mostly transmembrane proteins, including claudins, occludin, and junctional adhesion molecules (JAMs), which are stabilized by intracellular scaffolding protein, zona occludens (ZO) (Fig. 2) and further linked to actin filaments.[4,37] Occludin is the first identified protein of TJs that is a tetraspan integral membrane protein with both the N- and C- terminus located on the cytoplasmic side.[38] Occludin is involved in signal transduction and critical in maintaining the stability of TJs, and also highly phosphorylated at serine and threonine residues.[39,40] Occludin together with tricellulin and marvelD3 form the tight junction-associated MARVEL (MAL and related proteins for vesicle trafficking and membrane link) protein (TAMP) family. These proteins interact each other and have distinct but overlapping functions at TJs.[41] Claudins are the backbone of TJs and major players of epithelial TJs and paracellular barrier function. Claudins are integral membrane proteins containing four transmembrane domains with one intracellular loop, two extracellular loops, a cytoplasmic N-terminus and C-terminus.[40,42] Claudins interact with cytoplasmic PDZ scaffolding proteins such as ZOs through PDZ domain-binding motifs in its C- terminus domain.[43] Up to now, 27 members have been identified in the mammalian claudin family.[44] Each claudin member has a unique pattern of cell and tissue-specific distribution. Most members of the claudin family have TJ-tightening or barrier-forming properties; however, some claudin members, such as claudin 2, confer a pore-forming activity that is commonly associated with leaky epithelium.[45,46] The expression of claudin 2 is increased under different pathologic states such as IBD.[5,47,48] The structure, distribution and function of claudin-2 and other claudin family members have been comprehensively reviewed.[40,49]

JAMs are glycosylated transmembrane proteins with a single transmembrane domain, an extracellular N-terminus and a short cytoplasmic C-terminus.[50] They are widely distributed in various cell types and tissues [50] and have pleiotropic physiological functions, including epithelial barrier function, which has been previously reviewed.[40,51] JAMs function as cell-cell adhesion molecules through homophilic interactions with JAMs expressed by adjacent or opposing cells.[52]

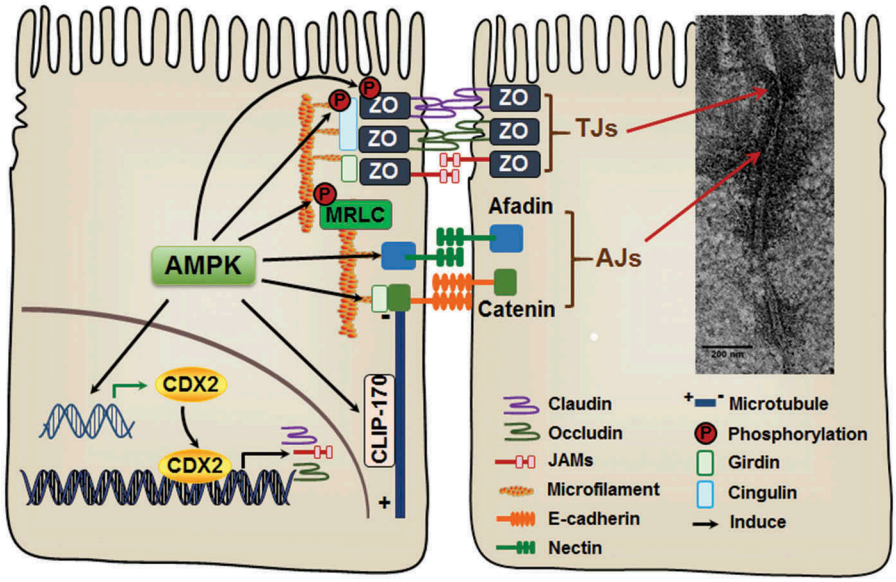


Figure 2. AMP-activated protein kinase (AMPK) activation accelerates the assembly of epithelial apical junctions. The apical side of epithelial cells is linked with adjacent epithelial cells through tight junctions (TJs) and adherens junctions (AJs). The primary constituents of TJs include transmembrane proteins, claudins, occludin, and junctional adhesion molecules (JAMs), which are stabilized by intracellular scaffolding protein zona occludens (ZO). AJs consist of two groups of transmembrane proteins, cadherins and nectins. Cadherins and nectins bind to the cytoskeleton by catenin-anchor proteins and afadin, respectively. AMPK activation accelerates TJ assembly stabilizes and maintains apical junctions, most likely through phosphorylation of TJ proteins and their associated proteins. In addition, AMPK enhances the expression of CDX2, which promotes epithelial differentiation and TJ protein expression.

JAM-A is a member of the JAM family highly expressed in intestinal epithelium.[53] JAM-A-deficient mice had enhanced intestinal permeability and were more susceptible to dextran sulfate sodium (DSS)-induced colitis,[6,7] while JAM-A content was decreased in intestinal mucosal extract of IBD patients compared to that from healthy subjects.[7] Overexpression of JAM-A enhanced barrier function in Caco-2 cells and ameliorated the barrier dysfunction caused by ethanol treatment.[53] The molecular structure, dimerization and functional motifs of JAM-A, and its interaction with scaffolding proteins, have been reviewed recently.[52]

Tricellulin is a 64-kD tetraspan, tricellular TJ protein commonly located at the region where three or more cells are adjacent,[54] and is involved in maintaining epithelial barrier integrity. Tricellulin is down-regulated in colon tissue biopsied from IBD, particularly from patients with ulcerative colitis,[55] as well as from enteropathogenic *E. coli* infected human colonic epithelial cells,[56] which are correlated with compromised barrier integrity.

Epithelial adherens junctions

AJs, located below TJs, maintain both the physical association between epithelial cells and cell polarity, and are involved in signal transduction. AJs consist of two groups of integral membrane proteins, cadherins and nectins. Cadherins and nectins bind to the cytoskeleton via catenin anchor proteins or afadin (Fig. 2).[57]

The cadherin superfamily contains of more than 20 members. Of these, epithelial cadherin (E-cadherin) is the most prominent in epithelial tissues and has a vital role in epithelial cell AJs assembly. The extracellular domain of E-cadherin contains five repetitive domains or cadherin repeats. Each cadherin repeat has a calcium-binding domain.[58] In the presence of calcium, E-cadherin interacts with other E-cadherins of the same or opposed cells through respective extracellular domains.[59] The cytoplasmic region of cadherin interacts with catenins and forms the cadherins-catenins complex, which then binds to actin microfilament (Fig. 2).[60] Similar to cadherins, nectins have a single transmembrane domain and interact with each other

through an extracellular domain.[61] The Inter-cellular interaction of nectins is not dependent on Ca^{2+}. Cytoplasmic tails of nectins interact with afadins through their PDZ binding motifs, which further associate with actin filaments (Fig. 2).[62] Nectins cooperate with cadherins in generating functional AJs.[57]

TJs and AJs are associated with each other physically via interaction between cytoplasmic scaffolding protein ZOs and catenins or afadins.[63,64] They are also linked through signaling molecules.[65] Ca^{2+} deprivation rapidly destroys and Ca^{2+} replenishment reinitiates TJ formation.[8] In addition, Ca^{2+} also drives AJ formation, which consolidates TJs,[66] suggesting that TJs and AJs both contribute to barrier integrity.

Favorite roles of AMPK in epithelial apical junctions

AMPK, a highly conserved serine/threonine kinase, regulates energy homeostasis to promote ATP generation and energy restoration when AMP/ATP ratio is elevated.[18] AMPK is composed of three subunits, including a catalytic α subunit, structural β subunit, and a regulatory γ subunit (Fig. 3). Under elevated AMP levels, the γ subunit binds to AMP, which renders AMPK a better phosphorylation substrate for its upstream kinase, liver kinase B (LKB1), at Thr 172,[67] and also inhibits its dephosphorylation.[68,69] AMPK can also be activated in response to the elevation of cellular Ca^{2+}, which involves its phosphorylation at Thr 172 by Ca^{2+}/calmodulin-dependent protein kinase kinase beta (CaMKKβ),[70] as well as transforming growth factor beta-activated kinase 1 (TAK1).[71] On the other hand, the phosphorylation of AMPK at Thr 172 can be removed by protein phosphatases such as protein phosphatase 2C alpha (PP-2Cα) (Fig. 3).[69] AMPK activity is commonly subjected to change in response to numerous physiological factors, such as hormones, cytokines, and dietary nutrients as well as pathological conditions, such as aging, metabolic syndrome, cancer and chronic inflammation.[72] Accumulating evidence has shown that AMPK plays an important role in determining gut epithelial health. Pharmacological and nutraceutical activation of AMPK strengthens epithelial barrier function,[8,25,27,28,73] while AMPK inhibition due to metabolic disorders is concurrent with impaired epithelial barrier function,[18,29] clearly showing the regulatory role of AMPK in epithelial permeability.

AMPK in tight junction formation and assembly

TJs assembly: TJ assembly of polarized epithelial cells is dynamic and critical in the formation and maintenance of the epithelial barrier. Extracellular Ca^{2+} is indispensable in TJs assembly, and AMPK is activated during Ca^{2+}-induced TJ assembly in MDCK

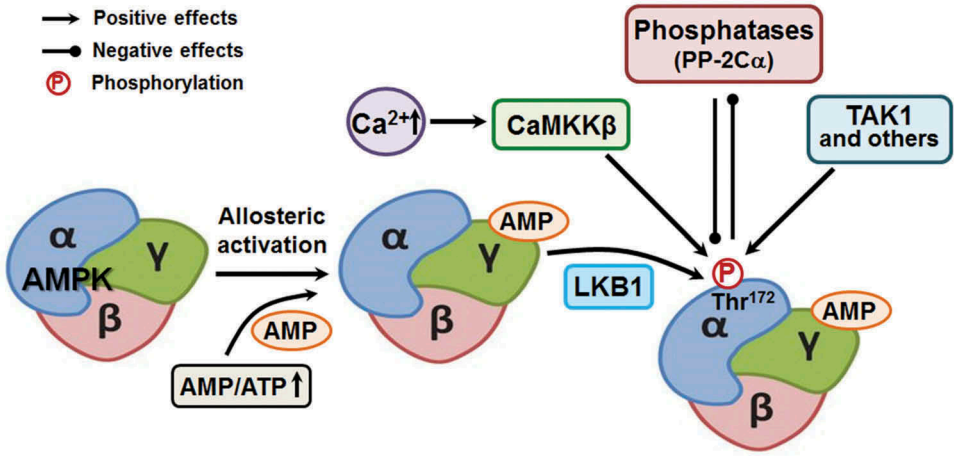


Figure 3. AMP-activated protein kinase (AMPK) structure and activation. AMPK is structurally composed of catalytic α subunit, docking β subunit and regulatory γ subunit. AMPK is phosphorylated/activated by LKB1, CaMKKβ, TAK1 and other kinases. CaMKKβ: calmodulin-mediated kinase kinase β; LKB1: liver kinase B1; PP-2Cα: protein phosphatase 2C alpha; TAK1: transforming growth factor-β activated kinase 1.

cells.[74,75] AMPK activation by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) enhanced the assembly of TJs while expression of AMPK kinase-dead constructs, K45R (AMPK α2 mutant) or D157A (AMPK α1 mutant) compromised TJ assembly as indicated by accelerated or delayed ZO-1 relocation to TJs and impaired transepithelial electrical resistance (TEER) in MDCK cells.[74,75] Furthermore, AICAR triggers and accelerates TJ assembly regardless of calcium availability,[74] suggesting AMPK activation promotes TJ formation independent of Ca^{2+}. [76] Also, in cultured MDCK cells, microvesicles derived from mesenchymal stromal cells facilitated Ca^{2+}-induced relocation of ZO-1 to TJ in an AMPK-dependent way.[77] Similarly, lymphocytes accelerated TJs assembly in MDCK cells following Ca^{2+} switch via AMPK activation.[78]

Likewise, in intestinal epithelial Caco-2 cells, ZO-1 relocation to TJs was enhanced in cells treated with AICAR or those with overexpression of AMPK wild-type plasmid, but was inhibited in cells expressing K45R AMPK mutant plasmid, which was associated with enhanced or compromised TEER and barrier function, respectively.[8] Consistently, the ultrastructure of TJs was greatly loosened, which was associated with increased intestinal permeability in AMPK VilCre mice where AMPK was specifically knocked out in villin-expressing epithelial cells.[8] Treatment with butyrate, a key metabolite of gut microbiota, activated AMPK and promoted TJ assembly in Caco-2 cells as indicated by enhanced ZO-1 and occludin redistribution, which was associated with enhanced TEER and decreased paracellular inulin permeability; however, Compound C (a cell-permeable AMPK inhibitor) treatment abolished butyrate-induced AMPK activation as well as barrier strengthening effect.[25] We recently reported that polyphenol-rich purple potato extract enhanced TEER in an AMPK- dependent manner and promoted ZO-1 relocation to TJs in Caco-2 cells.[27] Either chitosan oligosaccharides or flufenamic acid can promote TJ reassembly following Ca^{2+} switch and increase TEER in T84 cells in an AMPK-dependent manner, since co-incubation of either chitosan oligosaccharides or flufenamic acid with Compound C abolishes their beneficial effects on TJ assembly.[79,80]

Though the mechanism leading to AMPK-induced TJ assembly remains elusive, it might be regulated partially through direct phosphorylation of TJ proteins and associated proteins. AMPK phosphorylates claudin 1 at Thr191 that promotes the formation of TJs in EpH4 breast cells.[81] In submandibular SMG-C6 cells, AMPK activation phosphorylates claudin 4 at Ser199 and further enhances claudin 4 and occludin interaction.[82] Cytoskeleton microtubules are critical for the maintenance of TJ structure and function.[83] The elaborate interaction between apical junctions and cytoskeleton is essential for the maintenance and stabilization of TJs. Microtubules interact with TJs via cingulin anchored to claudin and occludin by ZO-1[84] Upon phosphorylation by AMPK at Ser132 and Ser150, cingulin connects microtubules to ZO-1, contributing to epithelial morphogenesis. AMPK inhibition distorts epithelial morphogenesis and detaches the network between micro-tubules and TJs,[84] showing a regulatory role of AMPK in anchoring of TJs to cytoskeleton. Girdin (also known as GIV), a polarity scaffold protein, preserves cell polarity and stabilizes TJs.[85] AMPK directly phosphorylates Girdin at Ser245, which is essential for maintaining apical junctional integrity and barrier function in MDCK cells.[85]

TJ protein content: In addition, AMPK can promote TJs through increasing TJ protein expression. Metformin supplementation increased barrier-promoting claudin 3 and decreased pore-forming caludin 2 content in the ileum tissue of interleukin (IL)-10-deficient mice, which was associated with improved barrier function.[73] In addition to strengthened TEER and decreased paracellular permeability, polyphenol-rich propolis extract[28] or purple potato extract [27] supplementation enhanced AMPK phosphorylation and the content of TJ proteins such as ZO-1, occludin and claudin1 in Caco-2 cells. Similarly, theaflavins promote barrier function in Caco-2 cells, likely through increasing TJ protein content, which was correlated with AMPK activation.[86] L-glutamine supplementation also promotes TEER and increases the content of TJ protein occludin, claudin 3, claudin 4, ZO-1, ZO-2 and ZO-3 as well as JAM-A in *in vitro* cultured intestine porcine epithelial cells, accompanied with AMPK

activation.[87] Butyrate strengthens intestinal barrier through enhancing TJ assembly instead of increasing TJ protein expression.[25] Conversely, AMPK intestinal specific deletion reduced ZO-1 content and impaired ZO-1 immunofluorescence staining at the tips of villi in jejunum tissue of AMPK Vilcre mice, (Fig. 4)[8].

AMPK in adherens junctions formation

AJs consist of two groups of transmembrane proteins, cadherins and nectins. The content of E-cadherin is upregulated in Caco−2 cells treated with AICAR, an AMPK activator, while downregulated in Caco−2 cells overexpressing K45R AMPK mutant or in jejunum tissues of mice with epithelium-specific AMPK knockout.[8] AMPK activation phosphorylates afadin, which induces Ca^{2+}-independent relocalization of AJ components,[76] providing a possible link between AMPK activation and apical junction stabilization. Metformin supplementation increased E-cadherin content in ileum tissue of both wild-type and IL-10-deficient mice, while E-cadherin content decreased in IL-10-deficient mice compared to wild-type mice.[73]

In addition, AMPK mediates cytoskeleton attachment of AJs. Microtubule organization is determined by proteins at both ends.[88] Microtubule plus-ends are dynamic, while minus-end proteins maintain stability.[89] CLIP-170, a microtubule plus-end binding protein, is phosphorylated by AMPK at Ser 311 to accelerate the microtubule polymerization and cell migration in renal 293T cells.[90] Non-muscle myosin regulatory light chain (MRLC) is an integrator driving cell adhesion and migration.[91,92] AMPK directly phosphorylates MRLC at Thr 21 in *Drosophila*.[93] The activation of AMPK due to 2-deoxyglucose-induced energy deprivation polarizes actin cytoskeleton in epithelial LS174T cells, which depends on MRLC phosphorylation by AMPK, [93] suggesting MRLC is involved in AMPK-dependent epithelial polarity establishment. AMPK is also a mediator of cell responses to mechanical force. In response to pulling forces applied to E-cadherin in breast MCF10A cells, AMPK is activated wit strengthens the cadherin-cytoskeleton complex to keep epithelial integrity.[24]

AMPK in epithelial differentiation, inflammation and barrier function

The establishment of apical junctions is positively related to epithelial differentiation.[94] AMPK promotes intestinal epithelial differentiation through promoting the expression of CDX2, a key transcription factor regulating epithelial differentiation.[8] Polyphenol-rich purple potato extract triggers

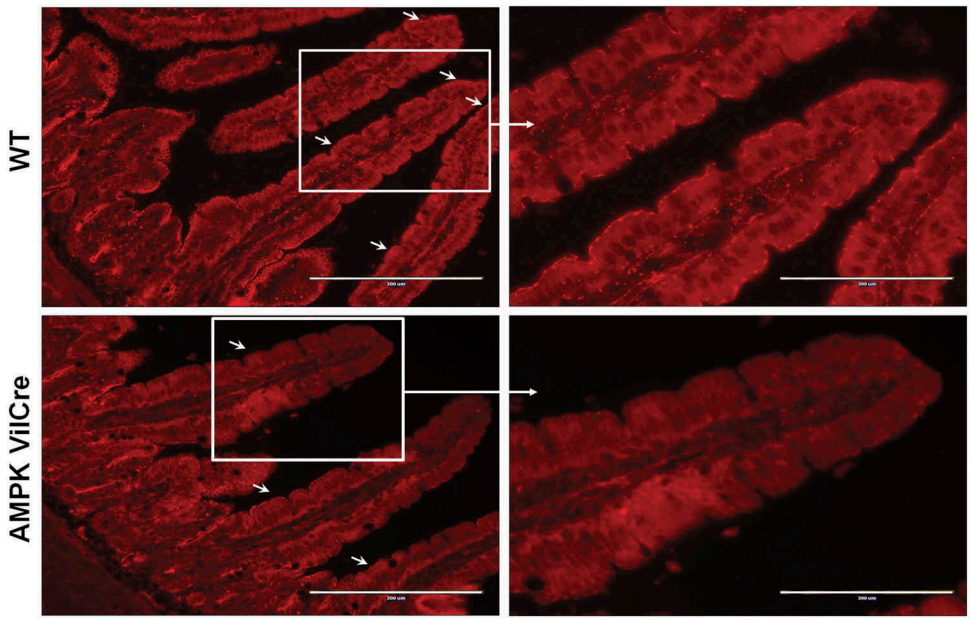
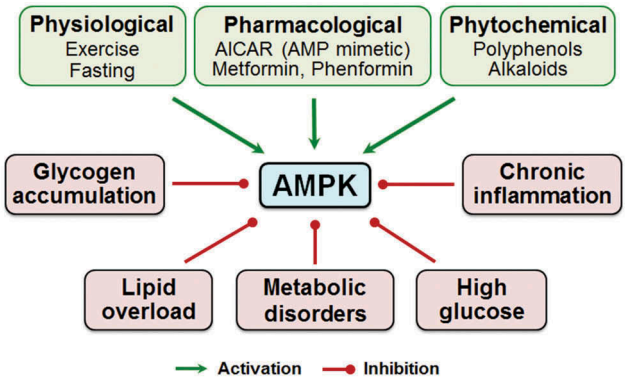


Figure 4. Immunofluorescent staining of ZO-1 in jejunum tissues of wild-type (WT) and AMPK VilCre knock out male mice at age of 10-week. Arrows indicate ZO-1 at the border of villus. Scale bar is 200 μm. Adopted from Sun et al., 2017 published in Cell Death & Differentiation.[8]



Phytochemicals activate AMPK		
Berberine	EGGC	Ginsenoside Rb1
Butyrate	Gallic acid	Quercetin
Curcumin	Genistein	Resveratrol

Figure 5. Physiological, nutritional and pharmacological factors regulate the activity of AMP-activated protein kinase (AMPK). AICAR: 5-aminoimidazole-4-carboxamide ribonucleoside; EGGC: Epigallocatechin gallate.

AMPK activation and increases expression of CDX2 in both Caco-2 cells and *ex vivo* guts. It also enhances barrier function and the epithelial differentiation markers villin, as well as brush border enzymes such as alkaline phosphatase, aminopeptidase and sucrose isomerase.[27] These beneficial effects were absent in Caco-2 cells with AMPK knockdown[27], indicating the beneficial effects of purple potato polyphenol extract on epithelial cell differentiation is mediated by AMPK. Besides transcription factors, epithelial differentiation is also regulated by complex signaling pathways. Bone morphogenetic protein (BMP) is one of the key pathways promoting intestinal differentiation.[95] Metformin supplementation decreases intestinal permeability, stimulates AMPK phosphorylation, and induces differentiation of goblet cells and Paneth cells in IL-10-deficient mice via AMPK-mediated BMP signaling pathway.[73]

Inflammation impairs epithelial barrier function.[26,96] Inflammation directly disrupts the assembly of apical junctions and thus increases epithelial permeability.[97] In addition, inflammation activates Wingless and Int (Wnt)/β-catenin signaling, which promotes epithelial proliferation but inhibits differentiation, thus impairing epithelial barrier formation.[96,98] AMPK activation triggers cell cycle arrest,[99,100] consistent with the repressive roles of AMPK in epithelial proliferation and promotion of differentiation. On the other hand, AMPK activation creates a pseudo-starving state that promotes oxidative metabolism and inhibits inflammation.[101] Metformin-induced AMPK activation reduces macrophage abundance, which suppresses mucosal inflammation and improves epithelial barrier function in IL-10-deficient mice.[73] Flufenamic acid or metformin supplementation protects barrier leakage in mouse ileum loop caused by *Vibrio cholera* infection. Either supplementation is associated with activation of AMPK and suppression of nuclear factor kappa B (NF-κB) inflammatory signaling and related inflammatory cytokine production.[80] Dietary red raspberry-activated AMPK attenuated inflammation and colitis symptoms in DSS-treated mice accompanied with reduced content of pore-forming claudin 2, and increased contents of barrier-strengthening TJ proteins.[47] Maternal high-fat diet enhanced offspring susceptibility to DSS-induced colitis, increased inflammatory cytokine IL-1β, IL-6 and IL-17, and amplified NF-κB inflammatory signaling response in mice, which was associated with suppressed AMPK phosphorylation.[102] Interestingly, in IL-10-deficient mice, AMPK phosphorylation is elevated, which could be due to energy dysregulation associated with chronic inflammation and oxidative stress; consistently, grape seed extract enriched with polyphenolic compounds prevented inflammation, and restored AMPK activity and barrier function in these mice.[96] In summary, AMPK improves gut epithelial barrier function through promoting oxidative metabolism and suppressing inflammation.

Perspectives and conclusion

AMPK activity is commonly subjected to change in response to physiological factors including hormones and cytokines, as well as pathological conditions, such as aging, metabolic syndrome, and chronic inflammation (Fig. 5).[72] Therefore, effectively restoring AMPK activity can serve as a therapeutic target to treat various metabolic diseases [103,104] and to improve various physiological functions, such as reinforcing apical junction assembly and gut barrier.

Metformin, a widely used medication for managing type 2 diabetes,[105] activates AMPK by increasing the cellular AMP level. Besides improving lipid

metabolism, metformin protects intestine epithelial barrier function in mice with fructose-induced steatosis[106] and in IL-10-deficient mice. [73] In addition to pharmacological drugs, many naturally occurring phytochemicals such as polyphenols and ginsenoside Rb1, a type of alkaloid isolated from ginseng, can effectively activate AMPK (Fig. 5).[107,108,109] Polyphenols, a diverse and abundant group of plant-derived bioactive compounds known for their anti-oxidative and anti-inflammatory properties, have preventive or therapeutic effects against metabolic diseases such as obesity, diabetes, aging, cardiovascular diseases and IBD.[110,111–114] The growing list of these compounds, including chlorogenic acid, curcumin, quercetin, resveratrol, and polyphenol-rich purple potato extract, exert their beneficial effects via activation of AMPK.[27,110,115–117] In addition, polyphenol-rich extracts and fruits [27,28,47] and gut microbial metabolite butyrate[25] activate AMPK and improve barrier function.

In summary, AMPK promotes the assembly and stability of apical junctions via the phosphorylation of TJ proteins and associated proteins. Additionally, AMPK promotes epithelial differentiation by upregulating epithelial transcription factors, suppressing Wnt/β-catenin signaling, and activating BMP signaling. Collectively, AMPK promotes epithelial barrier functions exerting health beneficial effects. The use of pharmacological and nutraceutical compounds, as well as the manipulation of physiological states triggering AMPK activation, are promising methods for strengthening epithelial barrier function and preventing metabolic diseases.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was reported by the authors.

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