AMP-activated protein kinase (AMPK), a highly conserved energy sensor, has a crucial role in cardiovascular, neurodegenerative and inflammatory diseases, as well as in cancer and metabolic disorders. Accumulating studies have demonstrated that AMPK activation enhances paracellular junctions, nutrient transporters, autophagy and apoptosis, and suppresses inflammation and carcinogenesis in the intestine, indicating an essential role of AMPK in intestinal health. AMPK inactivation is an aetiologi-

cal factor in intestinal dysfunctions. This review summarizes the favourable outcomes of AMPK activation on intestinal health, and discusses AMPK as a potential therapeutic target for intestinal diseases.

1. Introduction

The intestine is the longest and largest vital epithelial organ. Its major functions are absorbing nutrients from food and establishing selectively permeable barriers against the external environment [1]. To execute these functions, the intestinal epithelium needs to form a barrier, which depends on a well-balanced cellular homeostasis, orchestrated by a delicate interaction and balance among differentiation, self-renewal, proliferation and the intestinal ecosystem [2]. Disruption of the balance in intestinal homeostasis causes enteritis and colitis, leading to malnu-

trition, diarrhea, weight loss, constipation and fatigue [3,4]. Losses in intestinal homeostasis are associated with a broad range of pathological changes including metabolic disorders, inflammatory and autoimmune diseases, and cancers [5,6].

Adenosine monophosphate (AMP)-activated protein kinase (AMPK), a serine/threonine kinase, is evolutionarily conserved from yeast to mammals. As an energy sensor, AMPK is activated by upstream enzymes when the cellular ratio of AMP to adenosine triphosphate (ATP) is elevated due to nutrient depre-

vation [7]. After activation, AMPK phosphorylates downstream substrates to promote catabolism and impede anabolism, leading to ATP production and energy restoration [8,9]. AMPK activity can be altered owing to numerous phys-

ilological factors, such as hormones, cytokines and dietary nutrients, as well as pathological conditions such as obesity, chronic inflammation and type II dia-

betes [10]. Thus, AMPK activation can act as a therapeutic agent to treat various metabolic diseases [11,12]. Furthermore, the function of AMPK on the metabolism of liver and skeletal muscle has been well studied and documented [13,14]. AMPK also has a crucial role in regulating cellular development, such as adipogenesis [15], myogenesis [16] and osteogenesis [17].

Accumulating evidence supports the beneficial effects of AMPK on gut health, such as enhancing intestinal absorption [18], improving barrier function [19], suppressing colorectal carcinogenesis [20], and reducing intestinal inflamm-

ation [21] and metabolic-related disease [22]. Conversely, AMPK is inhibited under diabetic and obese conditions, which is correlated with impaired intestinal barrier function [23]. The inhibition of AMPK under pathological and physiologi-

cal states has been comprehensively discussed in a previous review [10]. In this review, we summarize the regulatory role of AMPK in intestinal health and
diseases, which links the important energy sensor to the maintenance of intestinal homeostasis.

2. AMPK and its regulators in the intestine

Intestinal mucosa contains the epithelial layer, the lamina propria, consisting mainly of connective tissue, and the muscularis mucosae layer, made of smooth muscle. AMPK is mainly located at the apical part of the villus epithelium in human adult jejunum [24]. AMPK is a heterotrimer, consisting of α catalytic subunits, and β and γ regulatory subunits. AMPK is activated by phosphorylation at Thr 172 of the α subunit by upstream kinases, such as liver kinase B1 (LKB1) and calmodulin-dependent protein kinase kinase (CaMKK) [25,26]. The binding of AMP to the γ subunit causes conformational changes which facilitate its phosphorylation by AMPK activators, such as LKB1 [27]. The α and β subunits each have two isoforms (α1 and α2, and β1 and β2), while the γ subunit has three isoforms (γ1, 2 and 3) [7]. The different heterotrimeric complexes display tissue specificity [24]. The AMPK α1/β2/γ1 complex is more abundant in differentiated intestinal epithelial cells [24]. Our recent study found that AMPK α1 deletion in intestinal epithelium suppresses intestinal differentiation in mouse jejunum with reduced mucosal height and villin content [19]. No change of epithelial architecture occurs in AMPKα2-deleted mice [28], which might be due to the pre-dominance of the α1 subunit in intestinal epithelium. The layers of connective tissue and smooth muscle tend to be thinner [28], which is probably due to the expression of the α2 subunit in non-epithelial cells. In addition, α1 is expressed during the initial stages in myogenesis, while α2 becomes dominant in differentiated myogenic cells [29], illustrating the tissue-specific expression of AMPK α isoforms.

AMPK is linked to the beneficial effects of nutraceutical or pharmacological compounds in intestinal health (table 1). 5-Aminooimidazole-4-carboxamide ribonucleotide (AICAR) is commonly used as a pharmacological activator for AMPK. It triggers AMPK activation through conversion into ZMP (Z refers to imidazole), an AMP analogue mimicking the AMP effect [62]. As expected, AMPK is activated in Caco-2 cells in response to AICAR treatment [24]. It has been shown that AICAR promotes intestinal glucose transportation [63] and barrier function [19], and inhibits infiltration of inflammatory cells [33]. Another pharmacological compound, metformin, a dimethylbiguanide, is a common anti-diabetic drug [64,65]. Metformin indirectly activates AMPK by inhibiting mitochondrial complex I in the respiratory chain to increase the AMP:ATP ratio [66]. In response to metformin, the phosphorylation of AMPK and its specific substrate acetyl-CoA carboxylase (ACC) increased 10-fold and 5-fold, respectively, in Caco-2 cells [24]. Metformin enhances intestinal glucose transportation [46] and inhibits inflammatory cytokines [67] and colitis [21]. Furthermore, microbial metabolite butyrate and other extracts from plants improve intestinal barrier function [38], suppress peptide transportation [42] and induce apoptosis in Caco-2 cells, associated with enhanced AMPK signalling. Though the mechanisms responsible for AMPK activation remain poorly defined, these plant-origin metabolites might inhibit mitochondrial function, including complex I in the respiratory chain and F1 ATP synthase, to increase the AMP:ATP ratio [63,68].

3. AMPK and intestinal absorption

The intestinal epithelium is composed of microvilli, villi and mucosal folds. To generate net influx, nutrients need to pass through the apical membrane of intestinal cells. Nutrients entering intestinal epithelial cells are either used by these cells or pass through the basolateral membrane of intestine cells into the circulatory system. The apical or basolateral transport can be energy-dependent (active transport with a carrier) or independent (passive transport). The passive transport depends on a concentration gradient. However, most of the sugars, amino acids, vitamins and minerals are transported by carriers or their respective transporters [69]. Thus, the functional regulation of intestinal transporters is critical for nutrient transportation.

Glucose is one of the most important nutrients in our body. Intestinal glucose absorption is mediated by glucose transporters, including glucose transporter 2 (GLUT2), glucose transporter 5 (GLUT5) and sodium–glucose transporter 1 (SGLT1) [70]. The temporal and quantitative regulation of glucose transporters governs glucose flux in and out of the intestine [71]. AMPK regulates glucose uptake not only in the heart [72], skeletal muscle [73], liver [74] and hippocampal neurons [75], but also in the gut. AMPKα2 knockout (KO) decreases protein levels of GLUT2 and GLUT5, while it increases protein levels of SGLT1 in the jejunum [28] (figure 1). Metformin increases GLUT3 expression [46], leading to translocation of GLUT2 to the apical membrane [18], which enhances glucose absorption in the gut [76,77]. Similarly, AICAR inhibits SGLT1 and promotes GLUT2 translocation in mice jejunal mucosa [18]. AMPK is upregulated in rats and pigs by feeding n-3 polyunsaturated fatty acids, which thereafter improve glucose uptake [52,53].

Ion transporters in intestinal epithelium are critical in keeping ion homeostasis [78]. Ion imbalance leads to diarrhoea. AMPK plays an important role in maintaining this homeostasis. Loss of AMPKα1 enhances epithelial sodium (Na+) channel (ENaC) expression, which controls the reabsorption of Na+ [79] (figure 1). After blocking ubiquitin ligase or endocytosis, phenformin is unable to block ENaC activity, suggesting that ubiquitin ligase is crucial in mediating the effects of AMPK on ENaC ubiquitination [79]. By activating AMPK with AICAR and phenformin in lung epithelial cells, ENaC expression is downregulated, which may be derived from the adaptation to metabolic stress to limit energy dissipation [80]. Apart from ENaC, AMPK also inhibits chloride (Cl−) secretion [56], as indicated by the reduction of ion-transport proteins and the cystic fibrosis transmembrane regulator (CFTR) [81] (figure 1). The overstimulation of Cl− secretion by CFTR is the predominant aetiology for enterotoxigenic diarrhoea [82]. AICAR and metformin, which activate AMPK, can offset the increased Cl− efflux by cholera toxin in excised intestinal loops [34], thus preventing diarrhoea. These studies suggest strong clinical applications for AMPK as a potential pharmacological target for treating acute diarrhoeal disease.

Peptide transporters mediate amino acid absorption [83]. AICAR attenuates the expression of peptide transporter 1 (PEPT1) [32], while the AMPK inhibitor Compound C promotes peptide transportation [42] (figure 1). AICAR inhibits apical dipeptide uptake in Caco-2 cells on trans-well filters [32]. The negative correlation between peptide absorption and AMPK activation might be due to the energy-dependent process of peptide uptake, which is dependent on Na+/K-ATPase...
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<th>compounds</th>
<th>functions</th>
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<tr>
<td>HCT116; HT-29; LoVo cells</td>
<td>adiponectin</td>
<td>inhibits cyclin E and cell growth; promotes p21, p27, glucose utilization and fatty acid oxidation</td>
<td>[30,31]</td>
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<tr>
<td>mice jejunum</td>
<td>AICAR</td>
<td>inhibits SGLT1; facilitates glucose transportation by GLUT2</td>
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<td>Caco-2 cells</td>
<td>AICAR</td>
<td>inhibits PEPT1</td>
<td>[32]</td>
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<td>Caco-2 cells</td>
<td>AICAR</td>
<td>promotes ZO-1 assembly and E-cadherin; enhances barrier function; inhibits intestinal permeability</td>
<td>[19]</td>
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<tr>
<td>mice colon</td>
<td>AICAR</td>
<td>promotes goblet cells; inhibits infiltration of inflammatory cells; downregulates macrophages</td>
<td>[33]</td>
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<td>Caco-2 cells; mice jejunum; human colonic mucosa</td>
<td>AICAR; metformin</td>
<td>inhibits chloride secretion</td>
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<tr>
<td>Caco-2 cells</td>
<td>alcohol</td>
<td>inhibits barrier function; disrupts cytoskeleton integrity</td>
<td>[35,36]</td>
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<td>HCT116; SW480; LOVO cells; mice colon</td>
<td>berberine</td>
<td>promotes goblet cells; inhibits infiltration of inflammatory cells; downregulates macrophages</td>
<td>[33]</td>
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<tr>
<td>Caco-2 cells</td>
<td>butyrate</td>
<td>enhances barrier function; facilitates ZO-1/occludin redistribution</td>
<td>[38]</td>
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<td>T84 cells; mice colon</td>
<td>chitosan oligosaccharide</td>
<td>promotes tight junction assembly; inhibits NF-κB transcriptional activity; prevents the development of aberrant crypt foci</td>
<td>[34,39]</td>
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<td>HT-29 cells</td>
<td>curcumin</td>
<td>induces COX-2</td>
<td>[40]</td>
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<td>combined 5-fluorouracil and genistein</td>
<td>induces COX-2</td>
<td>[41]</td>
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<tr>
<td>Caco-2 cells</td>
<td>Compound C</td>
<td>promotes PEPT1</td>
<td>[42]</td>
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<td>ECGG</td>
<td>induces COX-2</td>
<td>[20]</td>
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<tr>
<td>mice jejunum</td>
<td>leptin</td>
<td>promotes GLUT2 and GLUT5; decreases SGLT1</td>
<td>[28]</td>
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<td>mice jejunum and colon</td>
<td>high-fat diet</td>
<td>induces PPAR; triggers β-catenin activity; Increases intestinal tumorigenesis and villus length</td>
<td>[43,44]</td>
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<td>pig jejunum and ileum</td>
<td>lipopolysaccharide</td>
<td>decreases oleic acid, glutamine and glucose in enterocytes</td>
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<td>IL-10−/− mice colon</td>
<td>metformin</td>
<td>inhibits inflammatory cytokines and DSS-induced acute colitis</td>
<td>[21]</td>
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<td>COL0205 cells</td>
<td>metformin</td>
<td>inhibits IL-8 expression and NF-κB transcriptional activity</td>
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<td>rat small intestine</td>
<td>metformin</td>
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<td>metformin</td>
<td>facilitates localization of GLUT2 to apical membrane</td>
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<td>HCT116 xenografts</td>
<td>metformin</td>
<td>inhibits tumour growth lacking P53</td>
<td>[47]</td>
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<td>rat caecum</td>
<td>metformin</td>
<td>increases short chain fatty acid-producing bacteria</td>
<td>[48,49]</td>
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<td>rat duodenum</td>
<td>metformin</td>
<td>triggers GLP-1 from enteroendocrine L-cells; activates AMPK in hepatocytes in a non-autonomous manner</td>
<td>[50]</td>
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<td>Caco-2 cells</td>
<td>MIYAIRI 588</td>
<td>promotes ZO-1</td>
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<td>pig jejunum</td>
<td>n-3 polyunsaturated fatty acids</td>
<td>promotes glucose uptake</td>
<td>[52,53]</td>
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<td>pitavastatin</td>
<td>inhibits colonic preneoplastic lesions</td>
<td>[54,55]</td>
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<td>[54,56]</td>
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<td>propolis polyphenol</td>
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<td>[56,57]</td>
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<td>HT-29 cells</td>
<td>plumbagin</td>
<td>induces apoptosis via p53</td>
<td>[57,58]</td>
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<td>HT-29 cells</td>
<td>quercetin</td>
<td>induces apoptosis via p53</td>
<td>[58,59]</td>
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<td>HT-29 cells</td>
<td>selenium</td>
<td>induces COX-2</td>
<td>[59,60]</td>
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<td>Caco-2 cells</td>
<td>theaflavins</td>
<td>inhibits PEPT1</td>
<td>[42,60]</td>
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<td>HT-29 cells</td>
<td>20(S)-ginsenoside Rg3</td>
<td>induces apoptosis via p53</td>
<td>[42,61]</td>
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<td>pig jejunum and ileum</td>
<td>α-ketoglutarate</td>
<td>stimulates oxidation of energy substrates</td>
<td>[45,61]</td>
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differentiation. Green arrows indicate positive effects. Red lines indicate negative barriers. The gaps between adjacent cells are mechanically sealed by junctional complexes, including desmosomes, adherens junctions (AJs) and tight junctions (TJs) [96]. Tight junctions contribute to the selective paracellular permeability, while AJs are essential for TJ assembly [97]. Thus, intestinal barrier function depends on the performance of intestinal paracellular junctions, such as the establishment and reassembly of TJs, which is regulated by AMPK (figure 2).

In AMPK kinase-dead MDCK cells and Caco-2 cells, the formation of TJs (ZO-1) and establishment of transepithelial electric resistance (TEER) are delayed after calcium switch, while AICAR accelerates TJ reassembly [19,98]. Consistently, intestinal epithelium-specific deletion of AMPK α1 (AMPK VilCre) in mice enhanced intestinal permeability [19] (figure 1). Transmission electron microscopic observation further indicates that the ultrastructure of TJs is less compact in AMPK VilCre mice [19]. The paracellular junction is developed during intestinal differentiation [99], promoted by the intestinal transcription factor caudal type homeobox 2 (CDX2) [100]. AMPK promotes the expression of CDX2 and changes the CDX2 promoter-specific epigenetic modifications [19], providing a possible regulatory mechanism of AMPK on intestinal barrier function (figure 1). Interestingly, AMPK could be activated during TJ assembly via calcium switch, possibly due to the stimulated CaMKK (an AMPK activator) by an influx of calcium [98]. Furthermore, rapamycin rescues the delay of TJ assembly in AMPK kinase-dead cells, demonstrating that AMPK may, at least partially, mediate junction assembly via mammalian target of rapamycin (mTOR) signalling [98]. Additionally, AMPK profoundly promotes the formation of TJs in renal [101], mammary [102] and hepatic [103] epithelial cells.

Figure 1. AMPK promotes intestinal absorption and barrier function. AMPK regulates glucose absorption via enhancing the function of glucose transporter (GLUT)2 and GLUT5, while inhibiting sodium–glucose transporter 1 (SGLT1). AMPK mediates ion absorption through possible inhibition of cystic fibrosis transmembrane regulator (CFTR) and epithelial Na⁺ channel (ENaC). Peptide transporter 1 (PEPT1) expression is attenuated by AMPK to reduce apical dipeptide uptake. In addition, AMPK may phosphorylate myosin light chain kinase (MLCK) to enhance vasodilatation and blood flow, further favouring intestinal absorption. Besides absorbing nutrients, the intestine also functions as a frontier barrier protecting the mucosal integrity. AMPK facilitates the establishment of paracellular junctions (tight junctions and adherens junctions) via caudal type homeobox 2 (CDX2), an intestinal transcription factor to upregulate intestinal differentiation. Green arrows indicate positive effects. Red lines indicate negative effects. Solid lines represent proven regulations, while dashed lines represent possible regulations.

Figure 2. AMPK regulates intestinal inflammation and hormone secretion. AMPK suppresses intestinal inflammation through reducing pro-inflammatory cytokine production in macrophages, inhibiting the differentiation of T helper (Th) cells, promoting mucus secretion and enhancing autophagy, collaboratively. AMPK blocks the secretion of pro-inflammatory cytokines via inhibiting macrophage infiltration and differentiation of Th cells; AMPK triggers autophagy through activation of Ulk1-like autophagy activating kinase 1 (ULK1); AMPK increases goblet cells and associated mucus section, and enhances tight junctions (TJs) to strengthen intestinal barrier function. Gut microbiota and their metabolites such as short-chain fatty acids (SCFAs) regulate AMPK activation, exerting beneficial effects. Additionally, gut microbiota induces enterodocrine (EE) cells to generate the gut hormone ghrelin, leading to AMPK activation in the hypothalamus to increase food intake. On the other hand, the microbiota upregulates glucagon like peptide 1 (GLP-1) production from EE cells, which augments AMPK phosphorylation in the liver, subsequently reducing hepatic glucose production. Green arrows indicate positive effects; red lines indicate negative effects.
Many environmental factors impact intestinal barrier function associated with alteration of AMPK. Chitosan oligosaccharide and polyphenol extracts from Propolis upregulate TJ assembly and enhances the barrier function associated with AMPK activation [39,57]. The consumption of alcohol, a potent metabolic stressor, diminishes cellular ATP production and increases intestinal permeability [35,36]. AMPK activators such as metformin and AICAR exert ameliorative effects on disrupted barrier function caused by bacterial [104] and viral pathogens [105], and pro-inflammatory cytokines [106]. Epithelial barriers are dysfunctional in AMPK ViiCre mice [19], while metformin supplementation suppresses intestinal permeability, probably due to augmentation of epithelial differentiation [67].

Microbial metabolites, short-chain fatty acids (SCFAs), activate AMPK in colonocytes [38], associated with the enhanced TEER and redistribution of TJs [38]. Furthermore, butyrate protects against ethanol-induced intestinal barrier dysfunction, accompanied with AMPK activation [36]. Clostridium butyricum MIYAIRI 588, the butyrate-producing probiotic, enhances the activity of AMPK and strengthens TJs, resulting in mitigated gut permeability in non-alcoholic fatty liver disease [38,51]. Inhibition of AMPK either genetically or chemically abolishes the aforementioned therapeutic or prophylactic potential of SCFAs, demonstrating the modulatory role of AMPK in SCFAs’ enhanced barrier function [36].

5. AMPK and intestinal inflammation

The pathology of intestinal inflammation is mainly associated with immunological disorders [107]. Immune cells produce pro-inflammatory cytokines to defend against bacterial infections [108], triggering the activation of T cells and the recruitment of neutrophils [109]. Deficient regulatory T cells, excessive effector T cells and the overproduction of pro-inflammatory cytokines are all prone to inducing intestinal inflammation that exacerbates colitis [110,111]. Thus, the blocking of pro-inflammatory cytokines provides a therapeutic potential for inflammatory bowel disease (IBD), an autoimmune disease of the intestine [112].

Intestinal macrophages in the lamina propria are the primary source of pro-inflammatory cytokines [108]. The expression of inducible nitric oxide synthases (iNOSs) and tumour necrosis factor (TNFα) and phosphorylation of nuclear factor-kappa B (NF-κB) are inhibited in intestinal mucosal macrophages treated with AICAR [33]. In vitro, metformin suppresses TNFα-induced IL-8 expression and NF-κB inflammatory signalling [21], which facilitates T-cell differentiation [113]. AICAR suppresses the differentiation of Th1 and Th17 cells, possibly due to the downregulation of their transcriptional factors, T-bet and RORγt [33]. Lipopolysaccharides (LPSs), the major outer membrane component of Gram-negative bacteria, contribute to the inflammatory process of IBD [114]. LPS administration decreases phosphorylation of AMPK in pig jejenum and ileum (figure 2), which is ameliorated by dietary supplementation with α-ketoglutarate [45]. In addition, intraperitoneal injection with AICAR downregulates colonic macrophage activation in LPS-induced or 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis [33]. Thus, AMPK may provide an intervening target to ameliorate LPS-induced gut damage.

The mucus layer produced by goblet cells provides an additional protective barrier to the gut epithelium. The defective formation of the mucus layer increases mouse susceptibility to colitis [115], and colitis reduces the size and number of goblet cells in human gut [116]. AICAR supplementation augments goblet cell differentiation and attenuates the infiltration of inflammatory cells in TNBS-induced acute colitis [33] (figure 2).

The immune defences and repair systems are activated to maintain tissue integrity upon pathogen infection. Autoimmunity enhances cell survival under stress conditions, and keeps the balance between immunity and inflammation. This may play a protective role against inflammatory diseases such as IBD [117]. Unc-51-like autophagy activating kinase 1 (ULK1) is the earliest trigger for autophagocytosis, which is phosphorylated and binds with mTORC1 when nutrients are sufficient [118]. When nutrients are deprived, ULK1 dissociates from mTORC1, subsequently leading to initiation of autophagy [118]. AMPK involves ULK1-engaged autophagy by directly phosphorylating ULK1 at Ser 317 and Ser 777 to initiate autophagy [119] (figure 2).

Inflammation is closely related to metabolism. To synthesize ATP, cells can undergo either glycolysis or aerobic oxidation. Inflammatory cells, such as macrophages and T helper (Th) cells, typically undergo glycolytic metabolism [120]; on the other hand, anti-inflammatory cells typically depend on oxidative metabolism through mitochondria [120]. AMPK activation creates a pseudo-starving state that promotes oxidative metabolism and inhibits inflammation [120]. Similarly, creatine can regulate metabolism by recycling ATP in cells. Mutation in the creatine biosynthesis enzyme increases mice colitis, while creatine supplementation ameliorates colitis, possibly related to ATP supply and AMPK activation [121]. Collectively, these studies suggest that AMPK regulates intestinal inflammation partially through altering cell metabolism. AMPK activation shifts pro-inflammatory to anti-inflammatory cytokine production in macrophages, facilitates the differentiation of Th cells, and promotes epithelial barrier function and epithelial autophagy.

Up to now, most studies on the effects of AMPK on inflammation have been short-term studies. Limited information also points to the long-term effects of AMPK in suppressing inflammation. In an epidemiologic study, metformin suppresses chronic inflammation as indicated by the ratio of neutrophils to lymphocytes in the blood sample of diabetic patients [122]. Considering the commonness of chronic intake of AMPK activators, such as phenformin and metformin in diabetic patients, the long-term effect of AMPK activation on inflammation needs to be further investigated.

6. AMPK and colorectal cancer

Intestinal epithelium is the most dynamic tissue in the body, as it is constantly being renewed. Perturbations of the balance among proliferation, differentiation and apoptosis could result in the predisposition to and initiation of colorectal cancer (CRC), the third most lethal cancer in the United States [123,124]. Unlike normal epithelial cells, cancer cells depend on glycolysis to provide energy, the so-called Warburg effect [125]. As a metabolic mediator, AMPK is an anti-apoptotic component when cells are injured from glucose deprivation [126], hyperglycaemia [127], ceramide production [128] and
AMPK could also inhibit colorectal cancer through inhibiting cyclooxygenase-2 (COX-2), an inflammatory enzyme (figure 3). Epigallocatechin gallate (EGCG), a polyphenol derived from green tea, stimulates AMPK in a dose-dependent manner [20]. The activated AMPK thereafter inhibits the production of COX-2 and prostaglandin E2 to induce apoptosis, while Compound C abolishes it [20]. Similarly, curcumin [40], selenium [60], and combined 5-fluorouracil and genistein [41] all demonstrate anti-tumorigenic effects via the AMPK–COX2 cascade.

In addition, AMPK exerts anti-tumour effects through arresting the cell cycle and inducing apoptosis (figure 3). Adiponectin stimulates AMPK, arrests cell cycle progression at the G1 phase, reduces cyclin E, and stimulates p21, p27, glucose utilization and fatty acid oxidation [31]. AMPK arrests the cell cycle to inhibit proliferation in many established cancer cells including prostate cancer PC-3 [140], hepatoma HepG2 [141], brain C6 glioma, astrocytoma U87MG, T-lymphoblast CEM and breast cancer MCF-7 [142] cells.

CDX2 overexpression inhibits the growth and proliferation of colorectal cancer cells [143,144]. CDX2 expression is dramatically decreased during the late stages of malignant colorectal cancer [145]. CDX2 is absent in 183 out of 621 colorectal cancers from patient specimens [146]. Its loss is positively correlated with tumour grade and stage [146]. AICAR treatment upregulates CDX2 expression in Caco-2 cells, while CDX2 expression is dramatically downregulated in AMPKα2 KO Caco-2 cells and AMPK VilCre KO mice [19] (figure 3). CDX2 deletion abolished the promoted effects of AMPK on intestinal differentiation markers [19]. CDX2 mutation upregulates colonic polyp number and increases the proliferation of colonic cells [147], whereas the re-expression of CDX2 inhibits cyclin D1 expression and cell proliferation in human intestinal epithelial crypt cells lacking Cdx2 [148]. As CDX2 is a transcription factor that facilitates intestinal differentiation, enhancing differentiation could be a promising strategy for anti-cancer therapy.

The loss of epithelial polarity leads to the damage of intestinal organization, which is probably the major step for neoplastic transformation [149], subsequently resulting in epithelial—mesenchymal transition and metastasis [150]. It has been shown that AMPKα mutation in Drosophila embryos leads to abnormal distribution of epithelial polarity markers [151]. The consequent loss of polarity along with over-proliferative aberration elicits tumorous growths [152,153]. Therefore, the strengthened tight junction by AMPK provides a possible method for inhibiting adenocarcinoma and tumorigenesis (figure 3).

Chronic inflammation dramatically increases the risk of tumorigenesis. The reactive nitrogen intermediates and reactive oxygen species associated with inflammation usually trigger genomic instability and induce genetic mutations [154]. The DNA damage in turn initiates colorectal carcinogenesis. Intestinal inflammation is strongly associated with colon cancer, which has been comprehensively discussed by Terzic and co-authors [155]. AMPK suppresses many aspects of intestinal inflammation, which is discussed in §5 ‘AMPK and intestinal inflammation’. AMPK VilCre KO mice demonstrate exacerbated dextran sodium sulfate (DSS)-induced colitis [19], while metformin administration reduces colitis in interleukin-10-deficient mice [67] as well as DSS-induced colitis in mice [21]. AMPK might inhibit intestinal tumorigenesis through mitigating intestinal inflammation (figure 3).
7. Gut microbiota regulates AMPK activity

Gut microbiota show a close relationship with intestinal health [156]. Metagenomic analysis shows that the populations of Firmicutes and Bacteroidetes are profoundly reduced in the gut microbiota from IBD patients [157]. Bifidobacteria, Lactobacillus and Bacteroides ameliorate IBD, while Helicobacter hepaticus exacerbates IBD [156], probably due to their difference in SCFA production. SCFAs activate AMPK in colonocytes; both venous infusion and oral administration of SCFAs to mice activate AMPK [158], which may explain the regulatory effect of gut microbiota on AMPK activity (figure 2). Oral administration of metformin or berberine increases the population of Allobaculum, Bacteroides, Blautia, Butyricicoccus and Phascolarctobacterium in gut microbiota, which promotes SCFA production [48].

Besides activation by low energy level, AMPK can also be regulated by intestinal hormones in a cell non-autonomous manner [159,160] (figure 2). Prebiotic treatment enhances the generation of gut hormones, glucagon-like peptide (GLP)-1 and GLP-2, due to an increase in enteroendocrine L-cells in the colon of obese mice [161] (figure 2). Likewise, metformin triggers L-cells in rat duodenum to secrete GLP-1 [50]. GLP-1 enhances AMP and subsequently activates AMPK in hepatocytes to reduce hepatic glucose production in a non-autonomous manner [162]. However, AMPK mutation in hepatocytes abolished the beneficial effects of the gut-derived peptide GLP-1 [162].

Constant activation of AMPK in the hypothalamus is able to increase food intake and body weight, while AMPK inactivation reduces appetite in rodents [163,164]. Intraportal injection of ghrelin, the appetite-stimulating gastrointestinal hormone, upregulates rat food intake, associated with AMPK activation in presynaptic neurons [164,165] (figure 2). Consistently, AICAR injection into rat hypothalamus or cerebral ventricle enhances food intake [164], suggesting the integrative effect of AMPK in whole body metabolic regulation.

8. Conclusion

AMPK exerts protective effects on intestinal epithelial function through multiple mechanisms including improving intestinal absorption, enhancing barrier function, suppressing inflammation and preventing colorectal cancer. AMPK activation, either by pharmacological means or by nutraceuticals, might be a promising therapeutic strategy for treatment of intestinal disorders (figure 4).

Data accessibility. This article has no additional data.

Competing interests. We declare we have no competing interests.

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References


Aminoimidazole-4-carboxamide riboside induces intrinsic pathway relevant to activation of AMP-activated protein kinase. 


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**Gastroenterology** 1**3**8, 2101 – 2114.e5. (doi:10.1053/j.gastro.2010.01.058)

**Nat. Rev. Mol. Cell Biol.** 1**3**, 251 – 262. (doi:10.1038/nrm3311)