AMP-activated protein kinase: a therapeutic target in intestinal diseases

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Adenosine monophosphate (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 aetiological 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 malnutrition, diarrhoea, 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 deprivation [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 physiological factors, such as hormones, cytokines and dietary nutrients, as well as pathological conditions such as obesity, chronic inflammation and type II diabetes [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 inflammation [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 physiological 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 predominance 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-Aminoimidazole-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 dimethyl-biguanide, 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 transportation can be energy-dependent (active transport with a carrier) or independent (passive transport). The passive transportation 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 GLUT5 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

Table 1. Compounds targeting AMPK pathways in the intestine.

| experimental setting	compounds	functions	references
HCT116; HT-29; LoVo cells	adiponectin	inhibits cyclin E and cell growth; promotes p21, p27, glucose utilization and fatty acid oxidation	[30,31]
mice jejunum	AICAR	inhibits SGLT1; facilitates glucose transportation by GLUT2	[18]
Caco-2 cells	AICAR	inhibits PEPT1	[32]
Caco-2 cells	AICAR	promotes ZO-1 assembly and E-cadherin; enhances barrier function; inhibits intestinal permeability	[19]
mice colon	AICAR	promotes goblet cells; inhibits infiltration of inflammatory cells; downregulates macrophages	[33]
Caco-2 cells; mice jejunum; human colonic mucosa	AICAR; metformin	inhibits chloride secretion	[34]
Caco-2 cells	alcohol	inhibits barrier function; disrupts cytoskeleton integrity	[35,36]
HCT116; SW480; LOVO cells; mice colon	berberine	increases mTOR activity and p53 phosphorylation	[37]
Caco-2 cells	butyrate	enhances barrier function; facilitates ZO-1/Occludin redistribution	[38]
T84 cells; mice colon	chitosan oligosaccharide	promotes tight junction assembly; inhibits NF-κB transcriptional activity; prevents the development of aberrant crypt foci	[34,39]
HT-29 cells	curcumin	induces COX-2	[40]
HT-29 cells	combined 5-fluorouracil and genistein	induces COX-2	[41]
Caco-2 cells	Compound C	promotes PEPT1	[42]
HT-29 cells	EGCG	induces COX-2	[20]
mice jejunum	leptin	promotes GLUT2 and GLUT5; decreases SGLT1	[28]
mice jejunum and colon	high-fat diet	induces PPAR; triggers β-catenin activity; Increases intestinal tumorigenesis and villus length	[43,44]
pig jejunum and ileum	lipopolysaccharide	decreases oleic acid, glutamine and glucose in enterocytes	[45]
IL-10$^{-/-}$ mice colon	metformin	inhibits inflammatory cytokines and DSS-induced acute colitis	[21]
COLO205 cells	metformin	inhibits IL-8 expression and NF-κB transcriptional activity	[21]
rat small intestine	metformin	promotes GLUT5 expression	[46]
mice jejunum	metformin	facilitates localization of GLUT2 to apical membrane	[18]
HCT116 xenografts	metformin	inhibits tumour growth lacking P53	[47]
rat caecum	metformin	increases short chain fatty acid-producing bacteria	[48,49]
rat duodenum	metformin	triggers GLP-1 from enteroendocrine L-cells; activates AMPK in hepatocytes in a non-autonomous manner	[50]
Caco-2 cells	MIYAIRI 588	promotes ZO-1	[51]
Pig jejunum	n-3 polyunsaturated fatty acids	promotes glucose uptake	[52,53]
db/db mice colon	pitavastatin	inhibits colonic preneoplastic lesions	[54,55]
mice colon	phenformin	inhibits chloride secretion	[54,56]
Caco-2 cells	propolis polyphenol	promotes tight junctions; enhances the barrier function	[56,57]
HT-29 cells	plumbagin	induces apoptosis via p53	[57,58]
HT-29 cells	quercetin	induces apoptosis via p53	[58,59]
HT-29 cells	selenium	induces COX-2	[59,60]
Caco-2 cells	theaflavins	inhibits PEPT1	[42,60]
HT-29 cells	20(S)-ginsenoside Rg3	induces apoptosis via p53	[42,61]
pig jejunum and ileum	α-ketoglutarate	stimulates oxidation of energy substrates	[45,61]
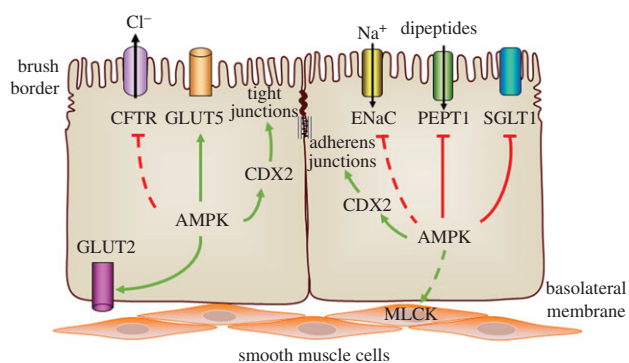
Figure 1. AMPK promotes intestinal absorption and barrier function. AMPK regulates glucose absorption via enhancing the function of glucose transporter (GLUT)2 and GLUT5, while inhibiting sodium–glucose transporter 1 (SGLT1). AMPK mediates ion absorption through possible inhibition of cystic fibrosis 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.

[84]. Thus, peptide transportation is suppressed under nutrient deprivation, probably accompanied by AMPK-mediated signalling pathways.

Another explanation for absorptive dysfunction due to AMPK inhibition is associated with impaired peristaltic activity of visceral musculature. It has been shown that AMPKα mutation in *Drosophila* impairs the movement of food through the digestive tract, which results in smaller fat body cells, delayed metamorphosis and growth inhibition [85]. As mesenteric circulation is directly proportional to nutrient transportation out of the intestine [86], the regulation of capillary blood flow contributes to intestinal absorption. AMPK stimulates vasodilatation and blood flow by attenuating contraction of vascular smooth muscle [87], possibly due to phosphorylation of myosin light chain kinase (MLCK) [88] (figure 1).

4. AMPK and intestinal barrier function

Proper intestinal barrier function plays a critical role in our health. Besides absorbing nutrients and secreting fluid, the intestine also functions as a critical barrier maintaining mucosal integrity, which physically inhibits the penetration of harmful substances from the external environment [89]. Impaired barrier function increases intestinal permeability to cause a leaky gut, predisposing individuals to intestinal bowel disease [90], metabolic syndromes [91–94] and autoimmune disorders [95].

The major determinant of gut epithelial permeability is closure or opening of paracellular junctions between enterocyte intercellular spaces [89]. 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
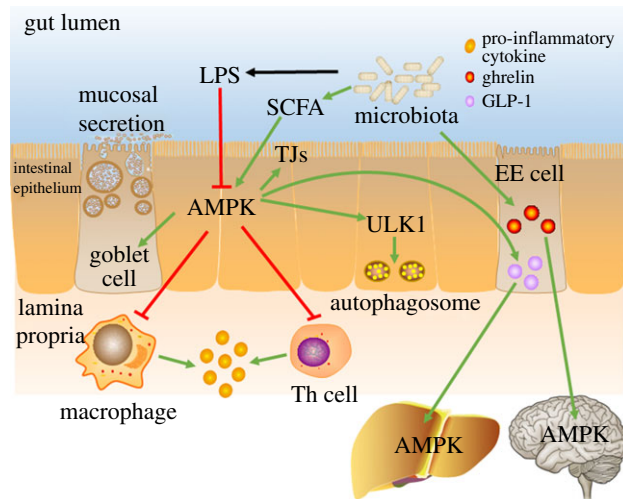

Figure 2. AMPK regulates intestinal inflammation and hormone secretion. AMPK suppresses intestinal inflammation through reducing pro-inflammatory cytokine production in macrophages, inhibiting the differentiation of T helper (Th) cells, promoting mucus secretion and enhancing autophagy, collaboratively. AMPK blocks the secretion of pro-inflammatory cytokines via inhibiting macrophage infiltration and differentiation of Th cells; AMPK triggers autophagy through activation of Unc-51-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 enteroendocrine (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.

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.

Many environmental factors impact intestinal barrier function associated with alteration of AMPK. Chitosan oligosaccharide and polyphenol extracts from Propolis upregulates 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 VilCre 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 (TNF)α 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 jejunum 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. Autophagy 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, those 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
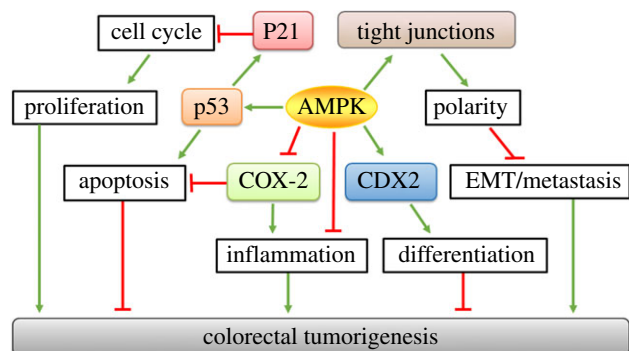
Figure 3. Suppressive mechanisms of AMPK in colorectal tumorigenesis. AMPK may suppress colorectal tumorigenesis through inhibiting proliferation, inflammation and metastasis as well as promoting apoptosis and differentiation collaboratively. AMPK induces apoptosis via p53 activation, which further upregulates p21 to impede cell cycling progression and cell proliferation; AMPK inhibits cancer progression by promoting caudal type homeobox 2 (CDX2) to induce differentiation, and by abating cyclooxygenase-2 (COX-2) to regulate apoptosis and inflammation; AMPK enhances the establishment of tight junctions to form epithelial polarity, which subsequently results in the amelioration of epithelial–mesenchymal transition (EMT) and colorectal metastasis. Green arrows indicate positive effects; red lines indicate negative effects.

ischaemia [129]. In contrast, AMPK induces apoptosis in pancreatic cells [130], gastric cancer cells [131] and neuroblastoma cells [132]. Thus, the effects of AMPK on apoptosis are dependent on cell type and external stimuli.

AMPK activation in the intestine is inhibited in metabolic diseases. In rats with insulin resistance, the protein content of phospho-AMPK is markedly decreased in the jejunum [24]. A high-fat diet (HFD) inactivates AMPK, and triggers β-catenin activity due to the induction of the peroxisome proliferator-activated receptor, which thereafter increases intestinal tumorigenesis and villus length [43,44]. Pitavastatin has chemopreventive potential on obesity-related colorectal carcinogenesis, associated with AMPK activation [54]. The adiponectin secreted by adipose tissue links metabolic diseases to tumorigenesis, probably mediated through AMPK. The incidence of colonic polyps increases in colorectal cancer with adiponectin deficiency, concomitantly with the inactivation of AMPK [133]. Consistently, growth inhibition by adiponectin in HT-29 cells is attenuated due to AMPK deficiency, and promoted when metformin is presented [30].

Mechanistically, AMPK could inhibit colorectal carcinogenesis through upregulating p53, a tumour suppressor (figure 3). Quercetin [59], the major dietary flavonoid, 20(S)-ginsenoside Rg3 from Panax ginseng [61] and plumbagin from plants [58] cause apoptosis via an AMPK–p53 cascade. Oral administration of quercetin reduces tumour volume and activates AMPK in mice with HT-29 tumour xenografts [59]. Activated AMPK phosphorylates p53 at Ser 15, which is partially responsible for the inhibition of tumour growth [134]. Furthermore, berberine activates AMPK, decreases mTOR activity and phosphorylates p53 in HCT116 cells [37]. Similar effects of quercetin were also detected in lung [135], breast [136], leukaemia [137] and prostate cancer cells [138]. As a tumour suppressor, p53 is often mutated in cancer. Of note, metformin and AICAR selectively inhibit tumour growth in HCT116 xenografts lacking p53, but not in those with intact p53 [139]. This may suggest a potential anti-tumour effect of metformin in patients with p53-deficient tumours.

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 G_1 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 overproliferative 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 Terzić 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]. Bifodobacteria, *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*, *Butyriciococcus* 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]. Intraperitoneal 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
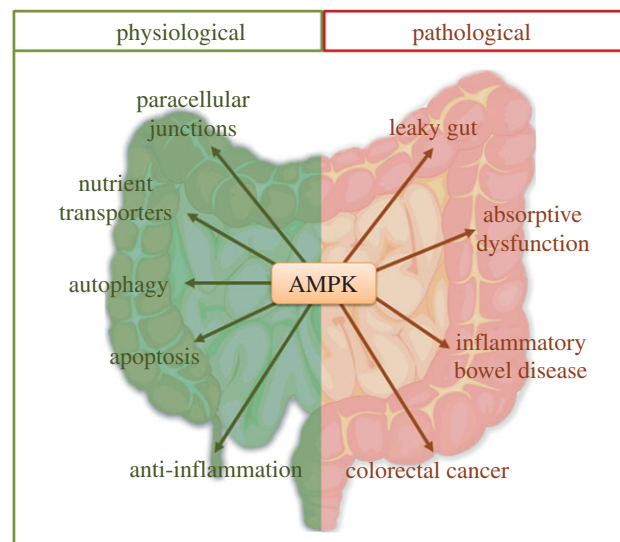

Figure 4. Implications and therapeutic perspectives of AMPK in the intestine. Under physiological conditions, AMPK activation strengthens paracellular junctions, enhances the function of nutrient transporters, promotes autophagy and apoptosis, and exerts anti-inflammatory effects. On the other hand, under pathological conditions, AMPK inactivation is implied in a number of intestinal diseases, such as leaky gut, absorptive dysfunction, inflammatory bowel disease and colorectal cancer.

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).

References

1. Stainier DY. 2005 No organ left behind: tales of gut development and evolution. *Science* **307**, 1902–1904. (doi:10.1126/science.1108709)

2. Moore KA, Lemischka IR. 2006 Stem cells and their niches. *Science* **311**, 1880–1885. (doi:10.1126/science.1110542)

3. Thompson WG. 1984 Gastrointestinal symptoms in the irritable bowel compared with peptic ulcer and inflammatory bowel disease. *Gut* **25**, 1089–1092. (doi:10.1136/gut.25.10.1089)

4. Talley NJ, Stanghellini V, Heading R, Koch K, Malagelada J, Tytgat G. 1999 Functional gastroduodenal disorders. *Gut* **45**(Suppl. 2), II37–II42.

5. Maloy KJ, Powrie F. 2011 Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* **474**, 298–306. (doi:10.1038/nature10208)

6. Rossi DJ, Jamieson CH, Weissman IL. 2008 Stems cells and the pathways to aging and cancer. *Cell* **132**, 681–696. (doi:10.1016/j.cell.2008.01.036)

7. Kahn BB, Alquier T, Carling D, Hardie DG. 2005 AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab.* **1**, 15–25. (doi:10.1016/j.cmet.2004.12.003)

8. Hutber CA, Hardie D, Winder W. 1997 Electrical stimulation inactivates muscle acetyl-CoA carboxylase and increases AMP-activated protein kinase. *Am. J. Physiol. Endocrinol. Metab.* **272**, E262–E266.

9. Hardie DG. 2007 AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat. Rev. Mol. Cell Biol.* **8**, 774–785. (doi:10.1038/nrm2249)

10. Viollet B, Horman S, Leclerc J, Lantier L, Foretz M, Billaud M, Giri S, Andreelli F. 2010 AMPK inhibition in health and disease. *Crit. Rev. Biochem. Mol. Biol.* **45**, 276–295. (doi:10.3109/10409238.2010.488215)

11. Brusq J-M, Ancellin N, Grondin P, Guillard R, Martin S, Saintillan Y, Issandou M. 2006 Inhibition of lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipidemic effects of berberine. *J. Lipid Res.* **47**, 1281–1288. (doi:10.1194/jlr.M600020-JLR200)

12. Chen MB, McAinch AJ, Macaulay SL, Castelli LA, O'Brien PE, Dixon JB, Cameron-Smith D, Kemp BE, Steinberg GR. 2005 Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle of obese type 2 diabetics. *J. Clin. Endocrinol. Metab.* **90**, 3665–3672. (doi:10.1210/jc.2004-1980)

13. Hardie DG, Sakamoto K. 2006 AMPK: a key sensor of fuel and energy status in skeletal muscle. *Physiology* **21**, 48–60. (doi:10.1152/physiol.00044.2005)

14. Ix JH, Sharma K. 2010 Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, and AMPK. *J. Am. Soc. Nephrol.* **21**, 406–412. (doi:10.1681/ASN.2009080820)

15. Hwang J-T, Park I-J, Shin J-I, Lee YK, Lee SK, Baik HW, Ha J, Pakr OJ. 2005 Genistein, EGCG, and capsaicin inhibit adipocyte differentiation process via activating AMP-activated protein kinase. *Biochem. Biophys. Res. Commun.* **338**, 694–699. (doi:10.1016/j.bbrc.2005.09.195)

16. Al-Khalili L, Chibalin AV, Yu M, Sjödin B, Nylén C, Zierath JR, Krook A. 2004 MEF2 activation in differentiated primary human skeletal muscle cultures requires coordinated involvement of parallel pathways. *Am. J. Physiol. Cell Physiol.* **286**, C1410–C1416. (doi:10.1152/ajpcell.00444.2003)

17. Kim EK, Lim S, Park JM, Seo JK, Kim JH, Kim KT, Ryu SH, Suh P-G. 2012 Human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by AMP-activated protein kinase. *J. Cell. Physiol.* **227**, 1680–1687. (doi:10.1002/jcp.22892)

18. Walker J, Jijon HB, Hugo D, Salehi P, Churchill T, Madsen KL. 2005 5-Aminoimidazole-4-carboxamide riboside (AICAR) enhances GLUT2-dependent jejunal glucose transport: a possible role for AMPK. *Biochem. J.* **385**, 485–491. (doi:10.1042/BJ20040694)

19. Sun X, Yang Q, Rogers CJ, Du M, Zhu M-J. 2017 AMPK improves gut epithelial differentiation and barrier function via regulating Cdx2 expression. *Cell Death Differ.* **24**, 819–831. (doi:10.1038/cdd.2017.14)

20. Hwang J-T, Ha J, Park I-J, Lee S-K, Baik HW, Kim YM, Park O. 2007 Apoptotic effect of EGCG in HT-29 colon cancer cells via AMPK signal pathway. *Cancer Lett.* **247**, 115–121. (doi:10.1016/j.canlet.2006.03.030)

21. Koh SJ, Kim JM, Kim IK, Ko SH, Kim JS. 2014 Anti-inflammatory mechanism of metformin and its effects in intestinal inflammation and colitis-associated colon cancer. *J. Gastroenterol. Hepatol.* **29**, 502–510. (doi:10.1111/jgh.12435)

22. Zhang BB, Zhou G, Li C. 2009 AMPK: an emerging drug target for diabetes and the metabolic syndrome. *Cell Metab.* **9**, 407–416. (doi:10.1016/j.cmet.2009.03.012)

23. Meddings J. 2008 The significance of the gut barrier in disease. *Gut* **57**, 438–440. (doi:10.1136/gut.2007.143172)

24. Harmel E *et al.* 2014 AMPK in the small intestine in normal and pathophysiological conditions. *Endocrinology* **155**, 873–888. (doi:10.1210/en.2013-1750)

25. Woods A *et al.* 2003 LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr. Biol.* **13**, 2004–2008. (doi:10.1016/j.cub.2003.10.031)

26. Woods A, Dickerson K, Heath R, Hong S-P, Momcilovic M, Johnstone SR, Carlson M, Carling D.

2005 Ca^{2+}/calmodulin-dependent protein kinase kinase-β acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab.* **2**, 21–33. (doi:10.1016/j.cmet.2005.06.005)

27. Suter M, Riek U, Tuerk R, Schlattner U, Wallimann T, Neumann D. 2006 Dissecting the role of 5′-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. *J. Biol. Chem.* **281**, 32 207–32 216. (doi:10.1074/jbc.M606357200)

28. Sakar Y, Nazaret C, Lettéron P, Ait Omar A, Avenati M, Viollet B, Ducroc R, Bado A. 2009 Positive regulatory control loop between gut leptin and intestinal GLUT2/GLUT5 transporters links to hepatic metabolic functions in rodents. *PLoS ONE* **4**, e7935. (doi:10.1371/journal.pone.0007935)

29. Fu X, Zhao J-X, Zhu M-J, Foretz M, Viollet B, Dodson MV, Du M. 2013 AMP-activated protein kinase α1 but not α2 catalytic subunit potentiates myogenin expression and myogenesis. *Mol. Cell. Biol.* **33**, 4517–4525. (doi:10.1128/MCB.01078-13)

30. Zakikhani M, Dowling RJ, Sonenberg N, Pollak MN. 2008 The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase. *Cancer Prev. Res.* **1**, 369–375. (doi:10.1158/1940-6207.CAPR-08-0081)

31. Kim AY *et al.* 2010 Adiponectin represses colon cancer cell proliferation via AdipoR1-and-R2-mediated AMPK activation. *Mol. Endocrinol.* **24**, 1441–1452. (doi:10.1210/me.2009-0498)

32. Pieri M, Christian HC, Wilkins RJ, Boyd C, Meredith D. 2010 The apical (hPepT1) and basolateral peptide transport systems of Caco-2 cells are regulated by AMP-activated protein kinase. *Am. J. Physiol. Gastrointest. Liver Physiol.* **299**, G136–GG43. (doi:10.1152/ajpgi.00014.2010)

33. Bai A, Ma AG, Yong M, Weiss CR, Ma Y, Guan Q, Bernstein CN, Perg Z. 2010 AMPK agonist downregulates innate and adaptive immune responses in TNBS-induced murine acute and relapsing colitis. *Biochem. Pharmacol.* **80**, 1708–1717. (doi:10.1016/j.bcp.2010.08.009)

34. Rogers AC *et al.* 2013 Activation of AMPK inhibits cholera toxin stimulated chloride secretion in human and murine intestine. *PLoS ONE* **8**, e69050. (doi:10.1371/journal.pone.0069050)

35. Manzo-Avalos S, Saavedra-Molina A. 2010 Cellular and mitochondrial effects of alcohol consumption. *Int. J. Environ. Res. Public Health* **7**, 4281–4304. (doi:10.3390/ijerph7124281)

36. Elamin EE, Masclee AA, Dekker J, Pieters H-J, Jonkers DM. 2013 Short-chain fatty acids activate AMP-activated protein kinase and ameliorate ethanol-induced intestinal barrier dysfunction in Caco-2 cell monolayers. *J. Nutr.* **143**, 1872–1881. (doi:10.3945/jn.113.179549)

37. Li W, Hua B, Saud SM, Lin H, Hou W, Matter MS, Jia L, Colburn NH, Young MR. 2014 Berberine regulates AMP-activated protein kinase signaling pathways and inhibits colon tumorigenesis in mice. *Mol. Carcinog.* **54**, 1096–1109. (doi:10.1002/mc.22179)

38. Peng L, Li Z-R, Green RS, Holzman IR, Lin J. 2009 Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of

AMP-activated protein kinase in Caco-2 cell monolayers. *J. Nutr.* **139**, 1619–1625. (doi:10.3945/jn.109.104638)

39. Muanprasat C, Wongkrasant P, Satitsri S, Moonwiriyakit A, Pongkorpsakol P, Mattaveewong T, Pichyangkura R, Chatsudthipong V. 2015 Activation of AMPK by chitosan oligosaccharide in intestinal epithelial cells: mechanism of action and potential applications in intestinal disorders. *Biochem. Pharmacol.* **96**, 225–236. (doi:10.1016/j.bcp.2015.05.016)

40. Lee YK, Park SY, Kim YM, Park OJ. 2009 Regulatory effect of the AMPK–COX-2 signaling pathway in curcumin-induced apoptosis in HT-29 colon cancer cells. *Ann. N. Y. Acad. Sci.* **1171**, 489–494. (doi:10.1111/j.1749-6632.2009.04699.x)

41. Hwang J-T, Ha J, Park OJ. 2005 Combination of 5-fluorouracil and genistein induces apoptosis synergistically in chemo-resistant cancer cells through the modulation of AMPK and COX-2 signaling pathways. *Biochem. Biophys. Res. Commun.* **332**, 433–440. (doi:10.1016/j.bbrc.2005.04.143)

42. Takeda J, Park H-Y, Kunitake Y, Yoshiura K, Matsui T. 2013 Theaflavins, dimeric catechins, inhibit peptide transport across Caco-2 cell monolayers via down-regulation of AMP-activated protein kinase-mediated peptide transporter PEPT1. *Food Chem.* **138**, 2140–2145. (doi:10.1016/j.foodchem.2012.12.026)

43. Mao J, Hu X, Xiao Y, Yang C, Ding Y, Hou N, Wang J, Cheng H, Zhang X. 2013 Overnutrition stimulates intestinal epithelium proliferation through β-catenin signaling in obese mice. *Diabetes* **62**, 3736–3746. (doi:10.2337/db13-0035)

44. Beyaz S *et al.* 2016 High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature* **531**, 53–58. (doi:10.1038/nature17173)

45. Hou Y *et al.* 2011 Effects of α-ketoglutarate on energy status in the intestinal mucosa of weaned piglets chronically challenged with lipopolysaccharide. *Br. J. Nutr.* **106**, 357–363. (doi:10.1017/S0007114511000249)

46. Lenzen S, Lortz S, Tiedge M. 1996 Effects of metformin on SGLT1, GLUT2, and GLUT5 hexose transporter gene expression in small intestine from rats. *Biochem. Pharmacol.* **51**, 893–896. (doi:10.1016/0006-2952(95)02243-0)

47. Wheaton WW *et al.* 2014 Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. *Elife* **3**, e02242. (doi:10.7554/eLife.02242)

48. Zhang X, Zhao Y, Xu J, Xue Z, Zhang M, Pang X, Zhang X, Zhao L. 2015 Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. *Sci. Rep.* **5**, 14405. (doi:10.1038/srep14405)

49. Shin N-R, Lee J-C, Lee H-Y, Kim M-S, Whon TW, Lee M-S, Bae J-W. 2013 An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* **63**, 727–735. (doi:10.1136/gutjnl-2012-303839)

50. Duca FA, Côté CD, Rasmussen BA, Zadeh-Tahmasebi M, Rutter GA, Filippi BM, Lam TKT. 2015 Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. *Nat. Med.* **21**, 506–511. (doi:10.1038/nm.3787)

51. Endo H, Niioka M, Kobayashi N, Tanaka M, Watanabe T. 2013 Butyrate-producing probiotics reduce nonalcoholic fatty liver disease progression in rats: new insight into the probiotics for the gut-liver axis. *PLoS ONE* **8**, e633880. (doi:10.1371/journal.pone.0063388)

52. Suchankova G, Tekle M, Saha AK, Ruderman NB, Clarke SD, Gettys TW. 2005 Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. *Biochem. Biophys. Res. Commun.* **326**, 851–858. (doi:10.1016/j.bbrc.2004.11.114)

53. Gabler NK, Radcliffe JS, Spencer JD, Webel DM, Spurlock ME. 2009 Feeding long-chain n-3 polyunsaturated fatty acids during gestation increases intestinal glucose absorption potentially via the acute activation of AMPK. *J. Nutr. Biochem.* **20**, 17–25. (doi:10.1016/j.jnutbio.2007.11.009)

54. Yasuda Y et al. 2010 Pitavastatin inhibits azoxymethane-induced colonic preneoplastic lesions in C57BL/KsJ-db/db obese mice. *Cancer Sci.* **101**, 1701–1707. (doi:10.1111/j.1349-7006.2010.01579.x)

55. Wang H, Zhao J-X, Hu N, Ren J, Du M, Zhu M-J. 2012 Side-stream smoking reduces intestinal inflammation and increases expression of tight junction proteins. *World J. Gastroenterol.* **18**, 2180. (doi:10.3748/wjg.v18.i18.2180)

56. Kongsuphol P, Hieke B, Ousingsawat J, Almaca J, Viollet B, Schreiber R, Kunzelmann K. 2009 Regulation of Cl⁻ secretion by AMPK *in vivo*. *Pflügers Arch.* **457**, 1071–1078. (doi:10.1007/s00424-008-0577-3)

57. Wang K, Jin X, Chen Y, Song Z, Jiang X, Hu F, Conlon M, Topping D. 2016 Polyphenol-rich propolis extracts strengthen intestinal barrier function by activating AMPK and ERK signaling. *Nutrients* **8**, 272. (doi:10.3390/nu8050272)

58. Chen M-B, Zhang Y, Wei M-X, Shen W, Wu X-Y, Yao C, Lu P-H. 2013 Activation of AMP-activated protein kinase (AMPK) mediates plumbagin-induced apoptosis and growth inhibition in cultured human colon cancer cells. *Cell. Signal.* **25**, 1993–2002. (doi:10.1016/j.cellsig.2013.05.026)

59. Kim H-J et al. 2010 Apoptotic effect of quercetin on HT-29 colon cancer cells via the AMPK signaling pathway. *J. Agric. Food Chem.* **58**, 8643–8650. (doi:10.1021/jf101510z)

60. Hwang J-T, Kim YM, Surh Y-J, Baik HW, Lee S-K, Ha J, Park OJ. 2006 Selenium regulates cyclooxygenase-2 and extracellular signal-regulated kinase signaling pathways by activating AMP-activated protein kinase in colon cancer cells. *Cancer Res.* **66**, 10 057–10 063. (doi:10.1158/0008-5472.CAN-06-1814)

61. Yuan H-d, Quan H-Y, ZHanG Y, KiM SH. 2010 20 (S)-Ginsenoside Rg3-induced apoptosis in HT-29 colon cancer cells is associated with AMPK signaling pathway. *Apoptosis* **2**, 6.

62. Corton JM, Gillespie JG, Hawley SA, Hardie DG. 1995 5-Aminoimidazole-4-carboxamide ribonucleoside. *Eur. J. Biochem.* **229**, 558–565. (doi:10.1111/j.1432-1033.1995.tb20498.x)

63. Hawley SA et al. 2010 Use of cells expressing γ subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab.* **11**, 554–565. (doi:10.1016/j.cmet.2010.04.001)

64. Mahmood K, Naeem M, Rahimnajjad NA. 2013 Metformin: the hidden chronicles of a magic drug. *Eur. J. Intern. Med.* **24**, 20–26. (doi:10.1016/j.ejim.2012.10.011)

65. Konopka AR et al. 2016 Hyperglucagonemia mitigates the effect of metformin on glucose production in prediabetes. *Cell Rep.* **15**, 1394–1400. (doi:10.1016/j.celrep.2016.04.024)

66. Doran E, Halestrap AP. 2000 Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem. J.* **348**, 607–614. (doi:10.1042/bj3480343)

67. Xue Y, Zhang H, Sun X, Zhu M-J. 2016 Metformin improves ileal epithelial barrier function in interleukin-10 deficient mice. *PLoS ONE* **11**, e0168670. (doi:10.1371/journal.pone.0168670)

68. Huang S-L, Yu R-T, Gong J, Feng Y, Dai Y-L, Hu F, Hu Y-H, Tao Y-D, Leng Y. 2012 Arctigenin, a natural compound, activates AMP-activated protein kinase via inhibition of mitochondria complex I and ameliorates metabolic disorders in ob/ob mice. *Diabetologia* **55**, 1469–1481. (doi:10.1007/s00125-011-2366-3)

69. Ferraris RP, Diamond JM. 1989 Specific regulation of intestinal nutrient transporters by their dietary substrates. *Annu. Rev. Physiol.* **51**, 125–141. (doi:10.1146/annurev.ph.51.030189.001013)

70. Wood IS, Trayhurn P. 2003 Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br. J. Nutr.* **89**, 3–9. (doi:10.1079/BJN2002763)

71. Thorens B. 1996 Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. *Am. J. Physiol. Gastrointest. Liver Physiol.* **270**, G541–GG53.

72. Russell RR, Bergeron R, Shulman GI, Young LH. 1999 Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. *Am. J. Physiol. Heart Circ. Physiol.* **277**, H643–H6H9.

73. Breen DM, Sanli T, Giacca A, Tsiani E. 2008 Stimulation of muscle cell glucose uptake by resveratrol through sirtuins and AMPK. *Biochem. Biophys. Res. Commun.* **374**, 117–122. (doi:10.1016/j.bbrc.2008.06.104)

74. Kawaguchi T, Osatomi K, Yamashita H, Kabashima T, Uyeda K. 2002 Mechanism for fatty acid 'sparing' effect on glucose-induced transcription. *J. Biol. Chem.* **277**, 3829–3835. (doi:10.1074/jbc.M107895200)

75. King TD, Song L, Jope RS. 2006 AMP-activated protein kinase (AMPK) activating agents cause dephosphorylation of Akt and glycogen synthase kinase-3. *Biochem. Pharmacol.* **71**, 1637–1647. (doi:10.1016/j.bcp.2006.03.005)

76. Kellett G, Helliwell P. 2000 The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. *Biochem. J.* **350**, 155–162. (doi:10.1042/bj3500155)

77. Au A, Gupta A, Schembri P, Cheeseman C. 2002 Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. *Biochem. J.* **367**, 247–254. (doi:10.1042/bj20020393)

78. Field M. 2003 Intestinal ion transport and the pathophysiology of diarrhea. *J. Clin. Invest.* **111**, 931. (doi:10.1172/JCI200318326)

79. Almaça J, Kongsuphol P, Hieke B, Ousingsawat J, Viollet B, Schreiber R, Amaral MD, Kunzelmanm K. 2009 AMPK controls epithelial Na+ channels through Nedd4-2 and causes an epithelial phenotype when mutated. *Pflügers Arch.* **458**, 713–721. (doi:10.1007/s00424-009-0660-4)

80. Woollhead AM, Scott JW, Hardie DG, Baines DL. 2005 Phenformin and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) activation of AMP-activated protein kinase inhibits transepithelial Na+ transport across H441 lung cells. *J. Physiol.* **566**, 781–792. (doi:10.1113/jphysiol.2005.088674)

81. Hallows KR. 2005 Emerging role of AMP-activated protein kinase in coupling membrane transport to cellular metabolism. *Curr. Opin. Nephrol. Hypertens.* **14**, 464–471. (doi:10.1097/01.mnh.0000174145.14798.64)

82. Cottreau J, Tucker A, Crutchley R, Garey KW. 2012 Crofelemer for the treatment of secretory diarrhea. *Expert Rev. Gastroenterol. Hepatol.* **6**, 17–23. (doi:10.1586/egh.11.87)

83. Leibach FH, Ganapathy V. 1996 Peptide transporters in the intestine and the kidney. *Annu. Rev. Nutr.* **16**, 99–119. (doi:10.1146/annurev.nu.16.070196.000531)

84. Kennedy D, Leibach F, Ganapathy V, Thwaites D. 2002 Optimal absorptive transport of the dipeptide glycylsarcosine is dependent on functional Na+/H+ exchange activity. *Pflügers Arch.* **445**, 139–146. (doi:10.1007/s00424-002-0910-1)

85. Bland ML, Lee RJ, Magallanes JM, Foskett JK, Birnbaum MJ. 2010 AMPK supports growth in *Drosophila* by regulating muscle activity and nutrient uptake in the gut. *Dev. Biol.* **344**, 293–303. (doi:10.1016/j.ydbio.2010.05.010)

86. McDaniel SS et al. 2001 Anorexic effect of K+ channel blockade in mesenteric arterial smooth muscle and intestinal epithelial cells. *J. Appl. Physiol.* **91**, 2322–2333.

87. Goirand F et al. 2007 Activation of AMP kinase α1 subunit induces aortic vasorelaxation in mice. *J. Physiol.* **581**, 1163–1171. (doi:10.1113/jphysiol.2007.132589)

88. Horman S et al. 2008 AMP-activated protein kinase phosphorylates and desensitizes smooth muscle myosin light chain kinase. *J. Biol. Chem.* **283**, 18 505–18 512. (doi:10.1074/jbc.M802053200)

89. Hollander D. 1999 Intestinal permeability, leaky gut, and intestinal disorders. *Curr. Gastroenterol. Rep.* **1**, 410 – 416. (doi:10.1007/s11894-999-0023-5)

90. Buhner S *et al.* 2006 Genetic basis for increased intestinal permeability in families with Crohn's disease: role of *CARD15* 3020insC mutation? *Gut* **55**, 342 – 347. (doi:10.1136/gut.2005.065557)

91. Miele L *et al.* 2009 Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* **49**, 1877 – 1887. (doi:10.1002/hep.22848)

92. Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, Martines D. 2007 Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **292**, G518 – GG25. (doi:10.1152/ajpgi.00024.2006)

93. Bosi E, Molteni L, Radaelli M, Folini L, Fermo I, Bazzigaluppi E, Piemonti L, Pastore MR, Paroni R. 2006 Increased intestinal permeability precedes clinical onset of type 1 diabetes. *Diabetologia* **49**, 2824 – 2827. (doi:10.1007/s00125-006-0465-3)

94. Teixeira TF, Collado MC, Ferreira CL, Bressan J, Maria do Carmo GP. 2012 Potential mechanisms for the emerging link between obesity and increased intestinal permeability. *Nutr. Res.* **32**, 637 – 647. (doi:10.1016/j.nutres.2012.07.003)

95. Jenkins R, Rooney P, Jones D, Bienenstock J, Goodacre R. 1987 Increased intestinal permeability in patients with rheumatoid arthritis: a side-effect of oral nonsteroidal anti-inflammatory drug therapy? *Rheumatology* **26**, 103 – 107. (doi:10.1093/rheumatology/26.2.103)

96. Farquhar MG, Palade GE. 1963 Junctional complexes in various epithelia. *J. Cell Biol.* **17**, 375 – 412. (doi:10.1083/jcb.17.2.375)

97. Schneeberger EE, Lynch RD. 2004 The tight junction: a multifunctional complex. *Am. J. Physiol. Cell Physiol.* **286**, C1213 – C1228. (doi:10.1152/ajpcell.00558.2003)

98. Zhang L, Li J, Young LH, Caplan MJ. 2006 AMP-activated protein kinase regulates the assembly of epithelial tight junctions. *Proc. Natl Acad. Sci. USA* **103**, 17 272 – 17 277. (doi:10.1073/pnas.0608531103)

99. Ichikawa-Tomikawa N, Sugimoto K, Satohisa S, Nishiura K, Chiba H. 2011 Possible involvement of tight junctions, extracellular matrix and nuclear receptors in epithelial differentiation. *Biomed. Res. Int.* **2011**, 253048. (doi:10.1155/2011/253048)

100. Silberg DG, Swain GP, Suh ER, Traber PG. 2000 Cdx1 and cdx2 expression during intestinal development. *Gastroenterology* **119**, 961 – 971. (doi:10.1053/gast.2000.18142)

101. Hallows KR, Mount PF, Pastor-Soler NM, Power DA. 2010 Role of the energy sensor AMP-activated protein kinase in renal physiology and disease. *Am. J. Physiol. Renal Physiol.* **298**, F1067 – F1F77. (doi:10.1152/ajprenal.00005.2010)

102. Yano T, Matsui T, Tamura A, Uji M, Tsukita S. 2013 The association of microtubules with tight junctions is promoted by cingulin phosphorylation by AMPK. *J. Cell Biol.* **203**, 605 – 614. (doi:10.1083/jcb.201304194)

103. Kojima T *et al.* 2009 Tight junction proteins and signal transduction pathways in hepatocytes. *Histol. Histopathol.* **24**, 1463 – 1472. (doi:10.14670/HH-24.1463)

104. Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S. 2003 Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. *Science* **300**, 1430 – 1434. (doi:10.1126/science.1081919)

105. Nava P, López S, Arias CF, Islas S, González-Mariscal L. 2004 The rotavirus surface protein VP8 modulates the gate and fence function of tight junctions in epithelial cells. *J. Cell Sci.* **117**, 5509 – 5519. (doi:10.1242/jcs.01425)

106. Utech M, Ivanov AI, Samarin SN, Bruewer M, Turner JR, Mrsny RJ, Parkos CA, Nusrat A. 2005 Mechanism of IFN-γ-induced endocytosis of tight junction proteins: myosin II-dependent vacuolarization of the apical plasma membrane. *Mol. Biol. Cell* **16**, 5040 – 5052. (doi:10.1091/mbc.E05-03-0193)

107. Bouma G, Strober W. 2003 The immunological and genetic basis of inflammatory bowel disease. *Nat. Rev. Immunol.* **3**, 521 – 533. (doi:10.1038/nri1132)

108. Kamada N *et al.* 2005 Abnormally differentiated subsets of intestinal macrophage play a key role in Th1-dominant chronic colitis through excess production of IL-12 and IL-23 in response to bacteria. *J. Immunol.* **175**, 6900 – 6908. (doi:10.4049/jimmunol.175.10.6900)

109. Sartor RB. 2006 Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat. Clin. Pract. Gastroenterol. Hepatol.* **3**, 390 – 407. (doi:10.1038/ncpgasthep0528)

110. Xavier R, Podolsky D. 2007 Unravelling the pathogenesis of inflammatory bowel disease. *Nature* **448**, 427 – 434. (doi:10.1038/nature06005)

111. Nielsen O, Kirman I, Rüdiger N, Hendel J, Vainer B. 2003 Upregulation of interleukin-12 and -17 in active inflammatory bowel disease. *Scand. J. Gastroenterol.* **38**, 180 – 185. (doi:10.1080/00365520310000672)

112. Neurath MF. 2017 Current and emerging therapeutic targets for IBD. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 269 – 278. (doi:10.1038/nrgastro.2016.208)

113. Gerondakis S, Fulford TS, Messina NL, Grumont RJ. 2014 NF-κB control of T cell development. *Nat. Immunol.* **15**, 15 – 25. (doi:10.1038/ni.2785)

114. Guo S, Al-Sadi R, Said HM, Ma TY. 2013 Lipopolysaccharide causes an increase in intestinal tight junction permeability *in vitro* and *in vivo* by inducing enterocyte membrane expression and localization of TLR-4 and CD14. *Am. J. Pathol.* **182**, 375 – 387. (doi:10.1016/j.ajpath.2012.10.014)

115. Kim YS, Ho SB. 2010 Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr. Gastroenterol. Rep.* **12**, 319 – 330. (doi:10.1007/s11894-010-0131-2)

116. Van Klinken BJ, Van der Wal JG, Einerhand A, Büller H, Dekker J. 1999 Sulphation and secretion of the predominant secretory human colonic mucin MUC2 in ulcerative colitis. *Gut* **44**, 387 – 393. (doi:10.1136/gut.44.3.387)

117. Randall-Demllo S, Chieppa M, Eri R. 2013 Intestinal epithelium and autophagy: partners in gut homeostasis. *Front. Immunol.* **4**, 301. (doi:10.3389/fimmu.2013.00301)

118. Yang Z, Klionsky DJ. 2010 Mammalian autophagy: core molecular machinery and signaling regulation. *Curr. Opin. Cell Biol.* **22**, 124 – 131. (doi:10.1016/j.ceb.2009.11.014)

119. Kim J, Kundu M, Viollet B, Guan K-L. 2011 AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132 – 141. (doi:10.1038/ncb2152)

120. O'neill LA, Hardie DG. 2013 Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature* **493**, 346 – 355. (doi:10.1038/nature11862)

121. Turer E, McAlpine W, Wang K-w, Lu T, Li X, Tang M *et al.* 2017 Creatine maintains intestinal homeostasis and protects against colitis. *Proc. Natl Acad. Sci. USA* **114**, E1273 – E1281. (doi:10.1073/pnas.1621400114)

122. Cameron AR *et al.* 2016 Anti-inflammatory effects of metformin irrespective of diabetes status. *Circ. Res.* **119**, 652 – 665. (doi:10.1161/CIRCRESAHA.116.308445)

123. Siegel RL, Miller KD, Jemal A. 2015 Cancer statistics, 2016. *CA Cancer J. Clin.* **66**, 7 – 30. (doi:10.3322/caac.21332)

124. Hanahan D, Weinberg RA. 2000 The hallmarks of cancer. *Cell* **100**, 57 – 70. (doi:10.1016/S0092-8674(00)81683-9)

125. Seyfried TN, Flores RE, Poff AM, D'Agostino DP. 2014 Cancer as a metabolic disease: implications for novel therapeutics. *Carcinogenesis* **35**, 515 – 527. (doi:10.1093/carcin/bgt480)

126. Culmsee C, Monnig J, Kemp BE, Mattson MP. 2001 AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation. *J. Mol. Neurosci.* **17**, 45 – 58. (doi:10.1385/JMN:17:1:45)

127. Ido Y, Carling D, Ruderman N. 2002 Hyperglycemia-induced apoptosis in human umbilical vein endothelial cells: inhibition by the AMP-activated protein kinase activation. *Diabetes* **51**, 159 – 167. (doi:10.2337/diabetes.51.1.159)

128. Peralta C *et al.* 2001 Adenosine monophosphate–activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion injury in the rat. *Hepatology* **34**, 1164 – 1173. (doi:10.1053/jhep.2001.29197)

129. RussellIII *et al.* 2004 AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. *J. Clin. Invest.* **114**, 495. (doi:10.1172/JCI19297)

130. Kefas BA, Cai Y, Kerckhofs K, Ling Z, Martens G, Heimberg H, Pipeleers D, Van de Casteele M. 2004 Metformin-induced stimulation of AMP-activated protein kinase in β-cells impairs their glucose responsiveness and can lead to apoptosis. *Biochem. Pharmacol.* **68**, 409 – 416. (doi:10.1016/j.bcp.2004.04.003)

131. Saitoh M, Nagai K, Nakagawa K, Yamamura T, Yamamoto S, Nishizaki T. 2004 Adenosine induces apoptosis in the human gastric cancer cells via an intrinsic pathway relevant to activation of AMP-activated protein kinase. *Biochem. Pharmacol.* **67**, 2005–2011. (doi:10.1016/j.bcp.2004.01.020)

132. Garcia-Gil M, Pesi R, Perna S, Allegrini S, Giannecchini M, Camici M, Tozzi MG. 2003 5′-Aminoimidazole-4-carboxamide riboside induces apoptosis in human neuroblastoma cells. *Neuroscience* **117**, 811–820. (doi:10.1016/S0306-4522(02)00836-9)

133. Sugiyama M *et al.* 2009 Adiponectin inhibits colorectal cancer cell growth through the AMPK/mTOR pathway. *Int. J. Oncol.* **34**, 339–344.

134. Motoshima H, Goldstein BJ, Igata M, Araki E. 2006 AMPK and cell proliferation—AMPK as a therapeutic target for atherosclerosis and cancer. *J. Physiol.* **574**, 63–71. (doi:10.1113/jphysiol.2006.108324)

135. Nguyen T, Tran E, Nguyen TH, Do P, Huynh T, Huynh H. 2004 The role of activated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. *Carcinogenesis* **25**, 647–659. (doi:10.1093/carcin/bgh052)

136. Singhal RL, Yeh YA, Prajda N, Olah E, Sledge G, Weber G. 1995 Quercetin down-regulates signal transduction in human breast carcinoma cells. *Biochem. Biophys. Res. Commun.* **208**, 425–431. (doi:10.1006/bbrc.1995.1355)

137. Shen SC, Chen YC, Hsu FL, Lee WR. 2003 Differential apoptosis-inducing effect of quercetin and its glycosides in human promyeloleukemic HL-60 cells by alternative activation of the caspase 3 cascade. *J. Cell. Biochem.* **89**, 1044–1055. (doi:10.1002/jcb.10559)

138. Lee H-J, Wang C-J, Kuo H-C, Chou F-P, Jean L-F, Tseng T-H. 2005 Induction apoptosis of luteolin in human hepatoma HepG2 cells involving mitochondria translocation of Bax/Bak and activation of JNK. *Toxicol. Appl. Pharmacol.* **203**, 124–131. (doi:10.1016/j.taap.2004.08.004)

139. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, Viollet B, Thompson CB. 2007 Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res.* **67**, 6745–6752. (doi:10.1158/0008-5472.CAN-06-4447)

140. Xiang X, Saha AK, Wen R, Ruderman NB, Luo Z. 2004 AMP-activated protein kinase activators can inhibit the growth of prostate cancer cells by multiple mechanisms. *Biochem. Biophys. Res. Commun.* **321**, 161–167. (doi:10.1016/j.bbrc.2004.06.133)

141. Imamura K, Ogura T, Kishimoto A, Kaminishi M, Esumi H. 2001 Cell cycle regulation via p53 phosphorylation by a 5′-AMP activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, in a human hepatocellular carcinoma cell line. *Biochem. Biophys. Res. Commun.* **287**, 562–567. (doi:10.1006/bbrc.2001.5627)

142. Rattan R, Giri S, Singh AK, Singh I. 2005 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside inhibits cancer cell proliferation *in vitro* and *in vivo* via AMP-activated protein kinase. *J. Biol. Chem.* **280**, 39 582–39 593. (doi:10.1074/jbc.M507443200)

143. Mallo GV, Soubeyran P, Lissitzky JC, Andre F, Farnarier C, Marvaldi J, Dagorn J-C, Lovanna JL. 1998 Expression of the Cdx1 and Cdx2 homeotic genes leads to reduced malignancy in colon cancer-derived cells. *J. Biol. Chem.* **273**, 14 030–14 036. (doi:10.1074/jbc.273.22.14030)

144. Suh E, Traber PG. 1996 An intestine-specific homeobox gene regulates proliferation and differentiation. *Mol. Cell. Biol.* **16**, 619–625. (doi:10.1128/MCB.16.2.619)

145. Ee HC, Erler T, Bhathal PS, Young GP, James RJ. 1995 Cdx-2 homeodomain protein expression in human and rat colorectal adenoma and carcinoma. *Am. J. Pathol.* **147**, 586–592.

146. Baba Y *et al.* 2009 Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. *Clin. Cancer Res.* **15**, 4665–4673. (doi:10.1158/1078-0432.CCR-09-0401)

147. Aoki K, Kakizaki F, Sakashita H, Manabe T, Aoki M, Taketo MM. 2011 Suppression of colonic polyposis by homeoprotein CDX2 through its nontranscriptional function that stabilizes p27Kip1. *Cancer Res.* **71**, 593–602. (doi:10.1158/0008-5472.CAN-10-2842)

148. Escaffit F, Paré F, Gauthier R, Rivard N, Boudreau F, Beaulieu J-F. 2006 Cdx2 modulates proliferation in normal human intestinal epithelial crypt cells. *Biochem. Biophys. Res. Commun.* **342**, 66–72. (doi:10.1016/j.bbrc.2006.01.128)

149. Sancho E, Batlle E, Clevers H. 2004 Signaling pathways in intestinal development and cancer. *Annu. Rev. Cell Dev. Biol.* **20**, 695–723. (doi:10.1146/annurev.cellbio.20.010403.092805)

150. Matter K, Aijaz S, Tsapara A, Balda MS. 2005 Mammalian tight junctions in the regulation of epithelial differentiation and proliferation. *Curr. Opin. Cell Biol.* **17**, 453–458. (doi:10.1016/j.ceb.2005.08.003)

151. Lee JH *et al.* 2007 Energy-dependent regulation of cell structure by AMP-activated protein kinase. *Nature* **447**, 1017–1020. (doi:10.1038/nature05828)

152. Bilder D. 2004 Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors. *Genes Dev.* **18**, 1909–1925. (doi:10.1101/gad.1211604)

153. Mirouse V, Swick LL, Kazgan N, St Johnston D, Brenman JE. 2007 LKB1 and AMPK maintain epithelial cell polarity under energetic stress. *J. Cell Biol.* **177**, 387–392. (doi:10.1083/jcb.200702053)

154. Grivennikov SI, Greten FR, Karin M. 2010 Immunity, inflammation, and cancer. *Cell* **140**, 883–899. (doi:10.1016/j.cell.2010.01.025)

155. Terzić J, Grivennikov S, Karin E, Karin M. 2010 Inflammation and colon cancer. *Gastroenterology* **138**, 2101–2114.e5. (doi:10.1053/j.gastro.2010.01.058)

156. Round JL, Mazmanian SK. 2009 The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* **9**, 313–323. (doi:10.1038/nri2515)

157. Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N, Pace NR. 2007 Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl Acad. Sci. USA* **104**, 13 780–13 785. (doi:10.1073/pnas.0706625104)

158. Carvalho B *et al.* 2012 Modulation of gut microbiota by antibiotics improves insulin signalling in high-fat fed mice. *Diabetologia* **55**, 2823–2834. (doi:10.1007/s00125-012-2648-4)

159. Minokoshi Y, Kim Y-B, Peroni OD, Fryer LG, Müller C, Carling D, Kahn BB. 2002 Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* **415**, 339–343. (doi:10.1038/415339a)

160. Yamauchi T *et al.* 2002 Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat. Med.* **8**, 1288–1295. (doi:10.1038/nm788)

161. Everard A *et al.* 2011 Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* **60**, 2775–2786. (doi:10.2337/db11-0227)

162. Ben-Shlomo S, Zvibel I, Shnell M, Shlomai A, Chepurko E, Halpern Z, Barzilai N, Oren R, Fishmsn S. 2011 Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. *J. Hepatol.* **54**, 1214–1223. (doi:10.1016/j.jhep.2010.09.032)

163. Minokoshi Y *et al.* 2004 AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* **428**, 569–574. (doi:10.1038/nature02440)

164. Andersson U, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR, Carling D, Small CJ. 2004 AMP-activated protein kinase plays a role in the control of food intake. *J. Biol. Chem.* **279**, 12 005–12 008. (doi:10.1074/jbc.C300557200)

165. Hardie DG, Ross FA, Hawley SA. 2012 AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol.* **13**, 251–262. (doi:10.1038/nrm3311)