

REVIEW

Regulation and function of AMPK in physiology and diseases

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5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) is an evolutionarily conserved serine/threonine kinase that was originally identified as the key player in maintaining cellular energy homeostasis. Intensive research over the last decade has identified diverse molecular mechanisms and physiological conditions that regulate the AMPK activity. AMPK regulates diverse metabolic and physiological processes and is dysregulated in major chronic diseases, such as obesity, inflammation, diabetes and cancer. On the basis of its critical roles in physiology and pathology, AMPK is emerging as one of the most promising targets for both the prevention and treatment of these diseases. In this review, we discuss the current understanding of the molecular and physiological regulation of AMPK and its metabolic and physiological functions. In addition, we discuss the mechanisms underlying the versatile roles of AMPK in diabetes and cancer.

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MOLECULAR REGULATION OF 5'-ADENOSINE MONOPHOSPHATE (AMP)-ACTIVATED PROTEIN KINASE (AMPK)

The basic and emerging molecular mechanisms of AMPK regulation are discussed below and summarized in Figure 1.

Basic mechanisms: adenylate charge, calcium and T172 phosphorylation

AMPK is a heterotrimeric complex containing one catalytic α -subunit and two regulatory β - and γ -subunits.¹ In mammals, AMPK α - and β -subunits have two isoforms each, and AMPK γ -subunit has three isoforms. This suggests the presence of 12 potential combinations of AMPK, each with different functions under different physiological conditions.² Several studies have suggested that these isoforms of AMPK subunits behave and are regulated differently under different physiological conditions.^{2,3} AMPK is regulated both allosterically and by post-translational modifications. The most well-defined mechanisms of AMPK activation are phosphorylation at T172 of the α -subunit and by AMP and/or adenosine diphosphate (ADP) binding to γ -subunit.⁴ Adenosine triphosphate (ATP) competitively inhibits the binding of both AMP and ADP to the γ -subunit, which suggests that AMPK is a sensor of AMP/ATP or ADP/ATP ratios.

Phosphorylation at T172 of the AMPK α -subunit is regulated by at least three kinases and three phosphatases: namely,

liver kinase B1 (LKB1), which exists in a heterotrimeric complex with STRAD and MO25; calcium-/calmodulin-dependent kinase kinase 2 (CaMKK2); TGF β -activated kinase 1 (TAK1); protein phosphatase 2A (PP2A); protein phosphatase 2C (PP2C) and Mg²⁺-/Mn²⁺-dependent protein phosphatase 1E (PPM1E).⁵⁻¹² In energy-replete conditions, that is, in the presence of low AMP/ATP and ADP/ATP ratios, phosphatases can easily access T172 of the AMPK α -subunit to keep it in the unphosphorylated state. However, when energy is depleted, high levels of AMP and ADP bind to CBS3 of the AMPK γ -subunit, which prevents the phosphatases from accessing T172 of the AMPK α -subunit, thus increasing its phosphorylation. In addition, binding of AMP and (to a lesser extent) ADP to CBS3 stimulates LKB1-mediated phosphorylation, which requires myristoylation of the AMPK β -subunit.¹³ Finally, the binding of AMP, but not of ADP, to CBS1 increases intrinsic AMPK activity by inducing its allosteric activation. In addition to the binding of adenylates, the binding of glycogen, especially glycogen with high branch points, to the β -subunit inhibits AMPK; however, the physiological significance of this is unclear.¹⁴ Intracellular calcium activates AMPK through CaMKK2-mediated phosphorylation. TAK1, a MAPKKK family member (MAP3K7), also phosphorylates and activates AMPK; however, the physiological conditions under which the TAK1-AMPK pathway operates remain to be elucidated.^{15,16}

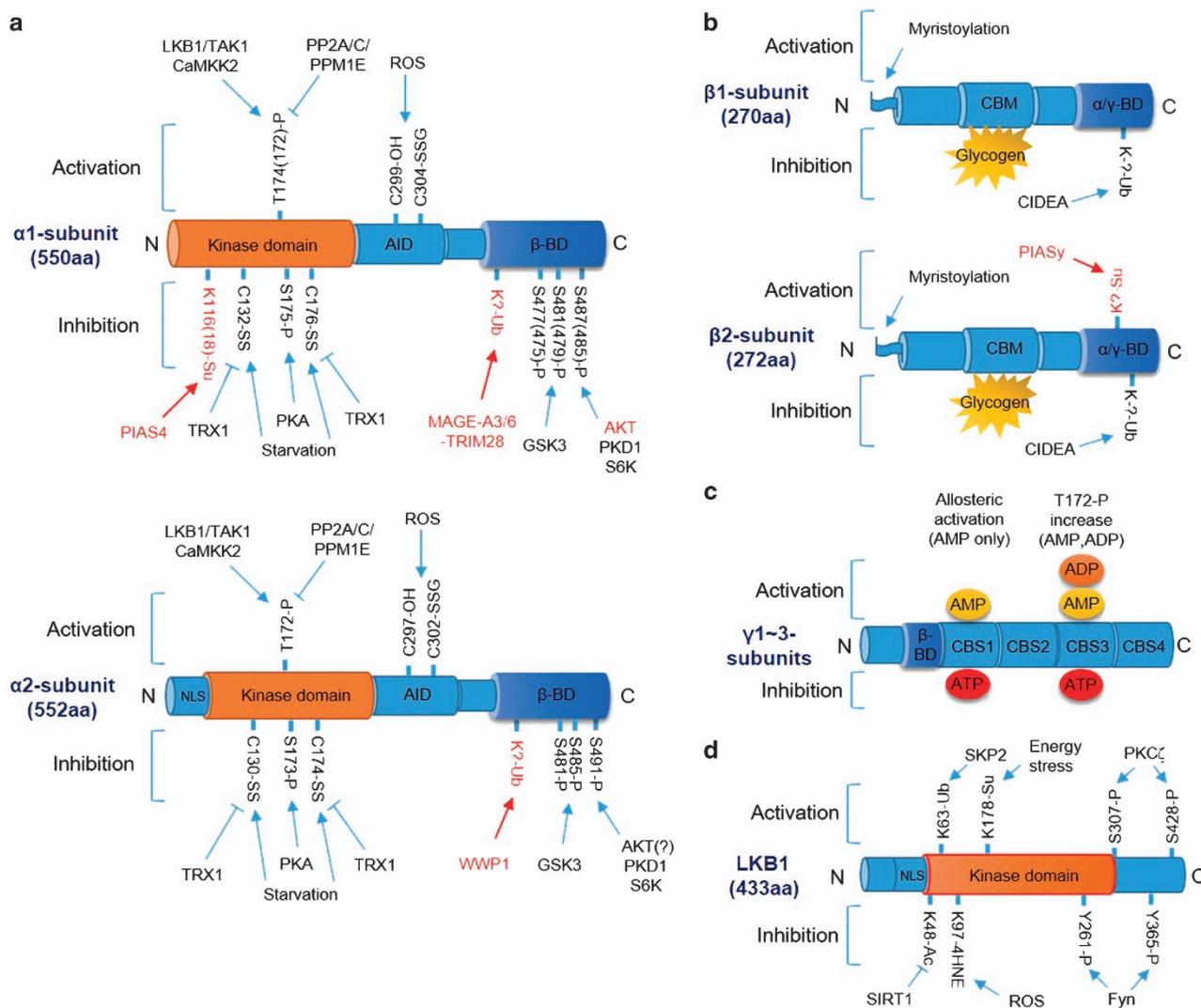


Figure 1 Molecular regulation of AMPK and LKB1. (a) Modification of the AMPK $\alpha 1$ (top) and $\alpha 2$ (bottom) subunits by phosphorylation/dephosphorylation, ubiquitination, sumoylation and oxidation/reduction. Pathways marked in red indicate $\alpha 1$ - or $\alpha 2$ -subunit-specific modifications. Numbers of modified amino acids are based on human proteins, and numbers in parenthesis are those reported in the original research (see text for details). (b) Modification of the AMPK $\beta 1$ (top) and $\beta 2$ (bottom) subunits by myristoylation, ubiquitination, sumoylation and glycogen binding. Pathways marked in red indicate $\beta 1$ - or $\beta 2$ -subunit-specific modifications (see text for details). (c) Modification of the AMPK γ -subunit by AMP, ADP or ATP binding. Binding of AMP to CBS1 induces allosteric activation, and binding of AMP or ADP to CBS3 induces T172 phosphorylation (see text for details). (d) Modification and regulation of LKB1 by phosphorylation, acetylation, ubiquitination, sumoylation and 4HNE adduction (see text for details). Arrow indicates activation, and bar-headed line indicates inhibition. α/γ -BD, α/γ -subunit-binding domain; AID, autoinhibitory domain; β -BD, β -subunit-binding domain; CBM, carbohydrate-binding module; CBS, cystathionine beta-synthase domain; NLS, nuclear localization signal.

Emerging mechanisms

Phosphorylation. Insulin inhibits AMPK by inducing its direct phosphorylation by AKT. AKT phosphorylates S485 of the AMPK $\alpha 1$ -subunit (S487 in humans) but does not phosphorylate an equivalent site in the AMPK $\alpha 2$ -subunit (S491), thus blocking upstream kinases from phosphorylating T172.¹⁷ Interestingly, a recent study showed that GSK3-induced phosphorylation at T479 of the AMPK $\alpha 1$ -subunit is required for the AKT-mediated inhibition of AMPK and vice versa, suggesting that an association between AKT and GSK3 is required for insulin-induced inhibition of AMPK.¹⁸ The satiety hormone leptin also inhibits AMPK by inducing p70S6K-dependent phosphorylation at S491

of the $\alpha 2$ -subunit.¹⁹ Furthermore, diacylglycerol (DAG), whose levels increase during hyperglycemia and hyperlipidemia, inhibits AMPK by inducing its direct phosphorylation by protein kinase C (PKC). A recent study showed that specifically PKD1 (PKC μ), one of the PKC isoforms, phosphorylates S491 of the AMPK $\alpha 2$ -subunit.²⁰ Finally, protein kinase A (PKA) also inhibits AMPK by phosphorylating S173 of the $\alpha 1$ -subunit, which blocks upstream kinases, such as LKB1 and CaMKK2, from phosphorylating T172.²¹

Ubiquitination/sumoylation. Ubiquitination inhibits AMPK by inducing its degradation. CIDEA, a cell death-inducing

DNA fragmentation factor 45-like effector (CIDE) family protein, interacts with and ubiquitinates the AMPK β -subunit in brown adipose tissue.²² However, the exact mechanisms underlying this are unclear. In addition, both the testis-restricted melanoma antigen MAGE-A3/6 and TRIM28 ubiquitin ligase complex ubiquitinate the AMPK α 1-subunit and are overexpressed in cancer cells, thus inducing its cancer-specific degradation.²³ WW domain-containing E3 ubiquitin protein ligase 1 also mediates the degradation of the AMPK α 2-subunit through ubiquitination in the presence of high glucose levels.²⁴ A recent study showed that SUMO E3 ligase PIAS4 catalyzes the sumoylation and inhibition of the AMPK α 1-subunit, which elicits specific activation of the mechanistic target of rapamycin complex 1 (mTORC1).²⁵ However, sumoylation by PIASy, which specifically targets the AMPK β 2-subunit, also activates AMPK and antagonizes its CIDEA-induced ubiquitination at the C-terminal of the β 2-subunit.²⁶

Oxidation. A growing body of evidence suggests that the reactive oxygen species (ROS) regulate AMPK activity, although the mechanism is controversial.^{27,28} Interestingly, recent studies showed that oxidative stress and energy stress differentially regulate AMPK activity through direct oxidation at cysteine residues in AMPK. In HEK293 cells, hydrogen peroxide activates AMPK through oxidation and S-glutathionylation at C299/C304 of its α -subunit.²⁹ However, the oxidation at C130/C174 of the AMPK α -subunit induced by H₂O₂ produced by glucose deprivation inhibits AMPK by promoting its aggregation and disrupting its interaction with upstream kinases.³⁰ The opposing effects of AMPK oxidation by different sources of ROS seem to be dependent on the abundance of nutrients and the antioxidant capacity of cells because energy stress-induced oxidation and inhibition of AMPK was reversed by the expression of antioxidant enzyme thioredoxin-1 (TRX1).³⁰

Protein–protein interactions/subcellular localization. AMPK activity can also be regulated by protein–protein interactions and its subcellular distribution. Although the mechanism is poorly understood, folliculin (FLCN), a tumor suppressor associated with Birt–Hogg–Dube syndrome, and its binding partners folliculin interacting protein-1 (FNIP-1) and -2 (FNIP-2) have been shown to interact with and inhibit AMPK.^{31,32} Interestingly, FLCN deficiency promotes metabolic transformation by activating the AMPK-peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC1 α)-hypoxia inducible factor 1 α axis.³³ The sestrin family of proteins interacts with AMPK and leads to the activation of the AMPK-TSC2 signaling axis to inhibit mTORC1.³⁴ In addition, upon AMP-dependent conformational change of AMPK, the scaffold protein axin promotes LKB1–AMPK complex formation, which enables efficient phosphorylation and activation of AMPK by LKB1 during energy stress.³⁵

N-myristoylated AMPK β -subunit serves as a scaffolding protein that recruits the AMPK α - and γ -subunits to intracellular membranes, including the mitochondrial membranes, in

response to energy stress.³⁶ The nuclear localization signal (NLS) present in the α 2-subunit, but not in the α 1-subunit, is critical for the nucleocytoplasmic translocation of AMPK, suggesting that α 1-subunit-containing AMPK mainly phosphorylates cytosolic substrates that regulate acute effects and that α 2-subunit-containing AMPK mainly phosphorylates transcriptional machinery that regulates gene expression for long-term effects.³⁷ In neurons, the AMPK γ 1-subunit preferentially localizes to the nucleus compared to other AMPK γ -subunits; however, the mechanisms underlying this translocation have not been explored to date.³⁸

LKB1 modification. The classic view suggests that LKB1 is constitutively active. However, accumulating data suggest that LKB1 activity is regulated by various physiological stimuli that induce post-translational modifications as summarized in Figure 1d. Because LKB1 contains an NLS domain but not a nuclear export domain, it is generally localized to the nucleus. LKB1 activation occurs after it complexes with MO25 and STRAD α , which induces its nuclear export and phosphorylation of its downstream targets, including AMPK, in the cytosol. LKB1 modifications that regulate its nucleocytoplasmic translocation for AMPK activation have been reported. PKC ζ phosphorylates S307/S428 (S399 for short form) of LKB1 and exports it to the cytosol, which is essential for AMPK activation.^{39–42} However, Fyn, a Src family non-receptor tyrosine kinase, phosphorylates Y261/Y365 of LKB1 to inhibit cytoplasmic translocation and AMPK activation.⁴³ Importantly, SIRT1, a class III NAD-dependent histone deacetylase, deacetylates K48 of LKB1 to induce its cytoplasmic localization and AMPK activation.⁴⁴ A recent study showed that metabolic stress triggers sumoylation at K178 of LKB1, which is essential for binding and phosphorylating AMPK.⁴⁵ Skp2-dependent ubiquitination at K63 of LKB1 activates LKB1 by maintaining the integrity of the LKB1–STRAD–MO25 complex and subsequently activates AMPK.⁴⁶ Interestingly, adduct formation between K97 of LKB1 and 4HNE, a lipid peroxidation marker, during oxidative stress inhibits LKB1, and in turn, AMPK.^{47,48}

METABOLIC FUNCTIONS OF AMPK

The key metabolic functions of AMPK are discussed below and summarized in Figure 2.

Lipid metabolism

The first known function of AMPK is the regulation of lipid metabolism. AMPK inhibits *de novo* synthesis of fatty acids (FAs), cholesterol and triglycerides (TGs), and activates FA uptake and β -oxidation (FAO). AMPK inhibits FA synthesis (FAS) by inducing the inhibitory phosphorylation of two targets: (1) acetyl-coA carboxylase 1 (ACC1), which catalyzes the rate-limiting step in FA synthesis by converting acetyl-coA to malonyl-coA, and (2) sterol regulatory element-binding protein 1c (SREBP1c), a transcription factor that promotes the expression of multiple lipogenic enzymes, including ACC1 and FA synthase.^{49,50} Excessive accumulation of FAs in cells are stored as TGs. The first committed step in TG synthesis is

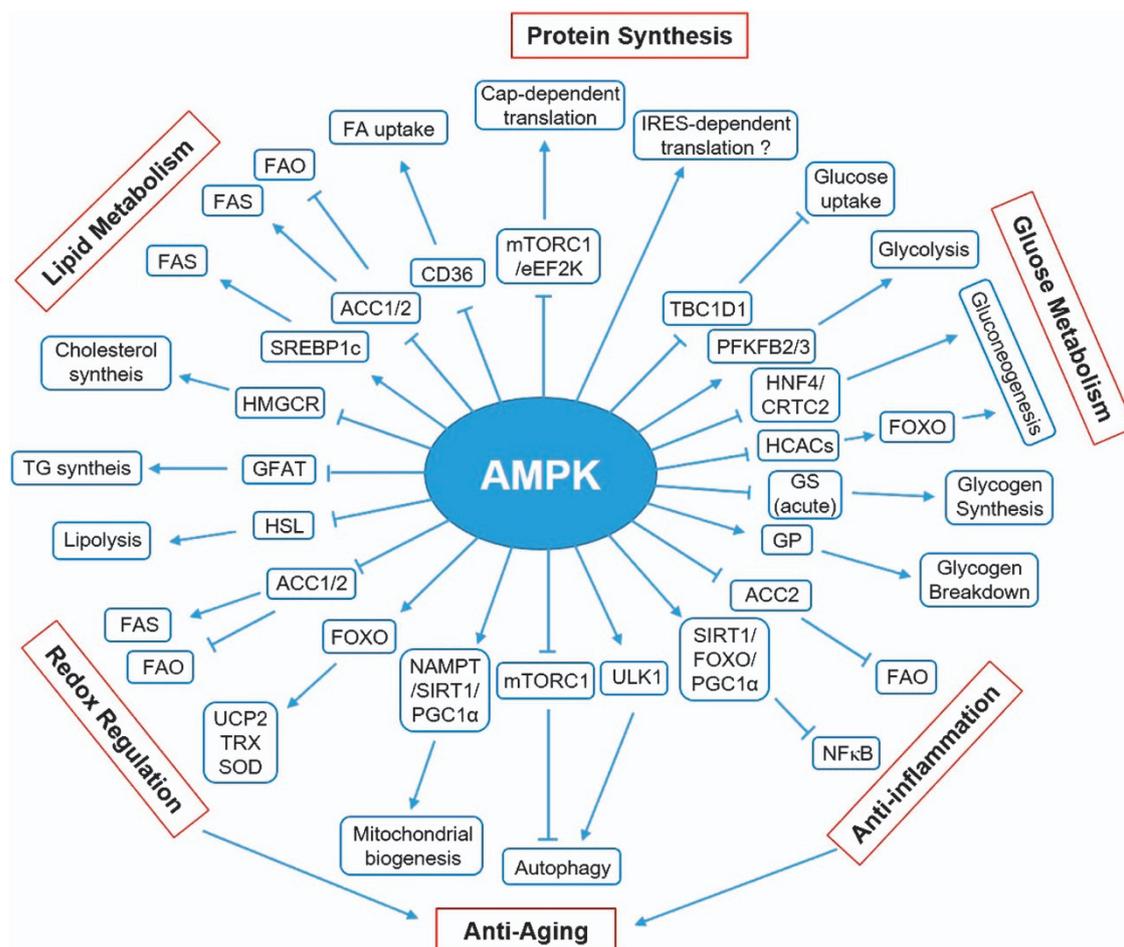


Figure 2 Metabolic functions of AMPK. A schematic summarizing the mechanisms underlying AMPK-induced regulation of diverse metabolic pathways. Arrow indicates activation, and bar-headed line indicates inhibition (see text for details).

catalyzed by glycerol-3-phosphate acyltransferase, which is inhibited by AMPK; however, it is unclear whether AMPK inhibits this step through direct phosphorylation or indirect regulation.⁵¹ AMPK also inhibits cholesterol synthesis by inducing the inhibitory phosphorylation of the rate-limiting enzyme HMG-CoA reductase.⁵²

In addition to the inhibition of lipid anabolism, AMPK activates lipid catabolism. AMPK increases FA uptake by controlling the translocation of FA transporter CD36 to the plasma membrane; however, the mechanism underlying this is unclear.⁵³ Once inside cells, FAs are transported into the mitochondria for β -oxidation by carnitine palmitoyltransferase-1 (CPT-1). AMPK increases CPT-1 activity and activates FAO by inducing the inhibitory phosphorylation of ACC2, which is localized to the outer membrane of the mitochondria near CPT-1 where it inhibits production of malonyl-CoA, a potent allosteric inhibitor of CPT-1.⁵⁰ However, AMPK inhibits lipolysis by inducing the inhibitory phosphorylation of hormone-sensitive lipase in the adipose tissue.⁵⁴ These data suggest that the central role of AMPK in lipid metabolism involves controlling the concentration of circulating free FAs (FFAs) by activating FAO, and by inhibiting lipolysis and lipogenesis.⁵⁵

Glucose metabolism

In skeletal muscles, AMPK stimulates glucose uptake by translocating GLUT4-containing intracellular vesicles across the plasma membrane. Fusion of these vesicles with the plasma membrane requires Rab family G proteins in their active GTP-bound state. AMPK phosphorylates and inhibits the Rab-GTPase-activating protein TBC1D1, which increases the activity of Rab family G proteins and induces fusion of GLUT4 vesicles with the plasma membrane.⁵⁶ Interestingly, a recent study showed that AMPK also increases glucose uptake in HEK293 cells by blocking endocytosis and by promoting GLUT1 expression.⁵⁷ AMPK-induced GLUT1 regulation is mediated by the phosphorylation and degradation of TRX-interacting protein, which induces GLUT1 internalization. AMPK also increases the mRNA expression of the genes encoding GLUT4 and hexokinase 2 to facilitate glucose uptake.^{58,59}

After glucose is transported into the cells, it is phosphorylated by hexokinases to generate glucose-6-phosphate. Glucose-6-phosphate is then consumed in several metabolic pathways including glycolysis, glycogen synthesis and pentose phosphate pathway. Among these pathways, glycolysis and glycogen synthesis are regulated by AMPK. Studies of cardiomyocytes,

macrophages/monocytes and tumor cells indicate that AMPK stimulates glycolysis by phosphorylating and activating 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2/3, which produces fructose-2,6-bisphosphate, an allosteric activator of glycolytic enzyme phosphofructokinase-1.^{60,61} AMPK inhibits glycogen synthesis through inhibitory phosphorylation of glycogen synthase (GS). However, chronic activation of AMPK can indirectly increase glycogen synthesis by increasing glucose uptake and glucose-6-phosphate production. This induces allosteric activation of GS that can overcome inhibitory phosphorylation by AMPK.⁶² Furthermore, AMPK also activates glycogen breakdown by phosphorylating and activating glycogen phosphorylase (GP).

Hepatic gluconeogenesis is important for maintaining blood glucose levels. AMPK inhibits gluconeogenesis by inhibiting several transcription factors, such as hepatocyte nuclear factor 4 (HNF4) and CREB regulated transcription coactivator 2 (CRTC2), that promote the expression of gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase and glucose-6-phosphatase.^{63,64} In addition, AMPK can also inhibit gluconeogenesis by phosphorylating and inducing the nuclear exclusion of class IIa histone deacetylases, which normally deacetylate and activate transcription factor FOXO in the nucleus, which results in the expression of gluconeogenic enzymes during fasting.⁶⁵

Protein synthesis

Protein synthesis is a high energy process that is inhibited during energy stress to conserve cellular ATP. AMPK inhibits cap-dependent translation during both initiation and elongation steps by indirectly inhibiting mTORC1 through the phosphorylation of TSC2 and raptor. Inhibition of mTORC1 activates 4EBP1 and inhibits p70S6K, thus inhibiting the initiation of cap-dependent translation and ribosomal proteins, respectively. AMPK also downregulates ribosomal RNA synthesis by inducing the inhibitory phosphorylation of transcription initiation factor 1A.⁶⁶ Furthermore, AMPK directly inhibits translational elongation by phosphorylating and activating eEF2K, which phosphorylates and inhibits eEF2.⁶⁷ However, expression of genes important for cell survival is also required during energy stress.⁶⁸ Interestingly, AMPK can perform these functions by switching translation from cap-dependent to cap-independent mechanisms. A recent study suggested that AMPK stimulates cap-independent and IRES-dependent translation of Hif-1 α during energy stress to induce the expression of genes critical for cell survival.⁶⁹

Autophagy and mitochondrial biogenesis

Autophagy is a lysosome-dependent self-digestive process that maintains cellular integrity during nutrient deficiency. Recent studies have shown that AMPK activates autophagy by directly and indirectly activating ULK1, a mammalian homolog of ATG1.^{70,71} First, AMPK directly phosphorylates and activates ULK1 to induce autophagy. Second, AMPK indirectly activates ULK1 by inhibiting mTORC1, which phosphorylates and inhibits ULK1 to disrupt the ULK1-AMPK interaction. This

coordinated regulation of ULK1 and mTORC1 eliminates damaged mitochondria and maintains mitochondrial integrity during nutrient starvation.^{70,71} In addition, the findings that FOXO upregulates expression of several autophagy inducers, such as Bnip3, LC3 and ATG12, and that AMPK phosphorylates and activates FOXO suggest that AMPK regulates autophagy by activating FOXO. Maintenance of mitochondrial integrity requires elimination of damaged mitochondria through autophagy and the production of fresh mitochondria through biogenesis. Moreover, autophagic degradation can contribute to energy generation by providing substrates for mitochondrial metabolism. Therefore, mitochondrial biogenesis is a crucial process for energy production and cellular response during nutrient deficiency. Pharmacological and genetic evidence indicates that AMPK regulates mitochondrial biogenesis by regulating PGC1 α , a cofactor that promotes the transcription of nuclear-encoded mitochondrial genes.⁷²⁻⁷⁴ At least four mechanisms have been proposed to be involved in AMPK-induced PGC1 α activation: namely, direct phosphorylation of PGC1 α ;⁷⁵ activation of SIRT1-mediated deacetylation of PGC1 α through an increase in NAD⁺/NADH ratio; AMPK-dependent increase in nicotinamide phosphoribosyltransferase (NAMPT) expression⁷⁶ or FAO⁷⁷ and p38 MAPK-dependent increase in PGC1 α expression.⁷⁸ SIRT1 activates AMPK by deacetylating LKB1,⁴⁴ which suggests the presence of a positive feedback loop between AMPK and SIRT1.

Redox regulation

Because metabolism is interconnected with redox regulation, AMPK has a crucial role in regulating antioxidant defense during oxidative stress. AMPK upregulates several antioxidant genes, such as those encoding superoxide dismutase and uncoupling protein 2, which reduce superoxide levels and thioredoxin (TRX), a disulfide reductase by phosphorylating and activating FOXO.^{79,80} Recent studies have suggested that NRF2, another transcription factor that is a master regulator of antioxidant response, is a potential target of AMPK to induce antioxidant defense.^{81,82} However, the mechanisms underlying this have yet to be elucidated. Furthermore, AMPK maintains NADPH levels by regulating FA metabolism through the phosphorylation and inhibition of ACC1/2 during metabolic stress.⁸³ FA synthesis is a major NADPH-consuming process. In contrast, FAO produces NADPH by increasing TCA cycle metabolites malate and isocitrate, which are catabolized by the NADPH-producing enzymes malic enzyme 1 (ME1) and isocitrate dehydrogenase 1 (IDH1), respectively. Inhibition of FAS and activation of FAO by the AMPK-ACC1/2 axis detoxifies ROS by maintaining NADPH and GSH levels. These data suggest that AMPK regulates antioxidant defense through both short- and long-term effects.

PHYSIOLOGICAL REGULATION OF AMPK

The physiological contexts that regulate AMPK activity and their physiological consequences are discussed below and summarized in Figure 3.

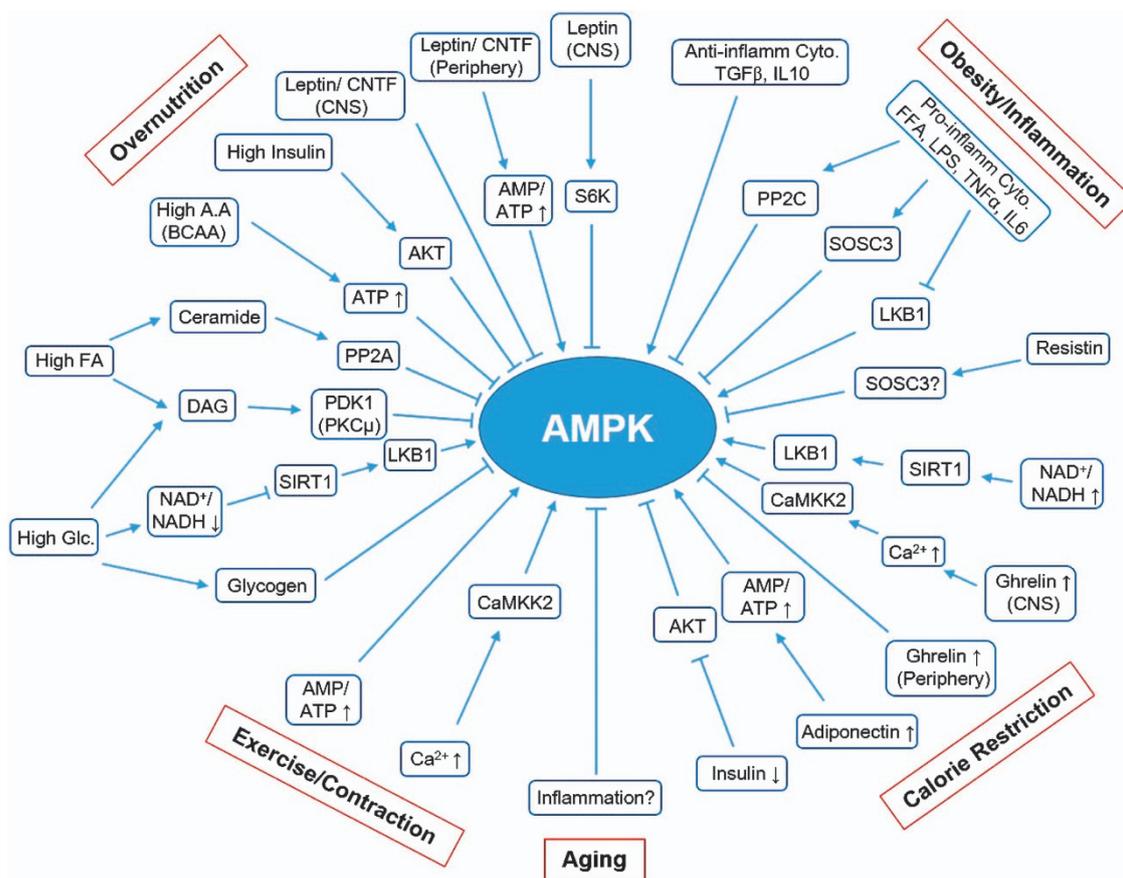


Figure 3 Physiological regulation of AMPK. A schematic summarizing the mechanisms underlying the regulation of AMPK activity under diverse physiological and pathological conditions. Arrow indicates activation, and bar-headed line indicates inhibition (see text for details).

Overnutrition

Accumulating data suggest that overnutrition and obesity are critical risk factors for modern chronic diseases, including insulin resistance, diabetes and cancer. Notably, the accumulation of three major nutrients, glucose, FAs and amino acids are suggested to suppress AMPK and contribute to insulin resistance.⁸⁴ High glucose levels inhibit AMPK through mechanisms that do not affect the AMP/ATP ratio.^{85–87} First, the reduced expression or indirect inhibition of SIRT1 by the reduction of NAD⁺/NADH ratio inhibits the SIRT1–LKB1 pathway. Second, high glucose levels induce the accumulation of DAG, a PKC activator that induces the inhibitory phosphorylation of S485/491 of the AMPK α -subunit. Moreover, glycogen accumulation and PP2A activation in the presence of high glucose inhibits AMPK. High levels of amino acids, especially branched-chain amino acids, inhibit AMPK by increasing ATP levels and by decreasing the AMP/ATP ratio; however, data on this mechanism are inconsistent.^{88–90} Excess saturated FAs inhibit AMPK by inducing the accumulation of DAG and ceramides, which can activate PKC and PP2A, respectively.⁹¹ Hyperinsulinemia accompanied by excessive nutrient accumulation inhibits AMPK by inducing AKT-mediated inhibitory phosphorylation at S485/491 of the AMPK α -subunit.⁹²

Leptin, the satiety and anti-obesity hormone secreted by adipocytes in the presence of insulin, prevents overnutrition by inhibiting AMPK in the hypothalamus to suppress appetite. In contrast, leptin activates AMPK in peripheral tissues, such as skeletal muscles, both directly by increasing the AMP/ATP ratio and indirectly through the hypothalamus–central nervous system axis involving the α -adrenergic signal.⁹³ However, mechanisms underlying leptin-induced differential regulation of AMPK in the hypothalamus and peripheral tissues have not been elucidated. Despite the important role of leptin in obesity control and insulin sensitization, leptin resistance has been reported in the skeletal muscles of obese individuals because of the upregulation of SOCS3, which prevents leptin-induced increase in the AMP/ATP ratio and activation of AMPK.⁹⁴ Similar to leptin, an anti-obesity cytokine ciliary neurotrophic factor suppresses appetite and activates peripheral FAO by differentially regulating AMPK.^{95,96} Moreover, cytokine ciliary neurotrophic factor differentially regulates AMPK and exerts its physiological effects even in leptin-resistant muscles, suggesting that it can be a promising therapeutic candidate for developing anti-obesity drugs.

Calorie restriction

Calorie restriction exerts many beneficial effects against aging, diabetes and cancer. In addition to its effect on the AMP/ATP

ratio, calorie restriction activates AMPK through multiple mechanisms. First, calorie restriction increases the NAD^+/NADH ratio, which activates SIRT1, which in turn activates AMPK by deacetylating and activating LKB1.^{44,97} Second, as opposed to overnutrition discussed above, calorie restriction decreases blood insulin levels that may activate AMPK by decreasing its AKT-mediated inhibitory phosphorylation.⁸⁴ Third, calorie restriction stimulates adiponectin secretion from adipocytes that activates AMPK in multiple tissues, including skeletal muscles.⁹⁸ Interestingly, adiponectin secretion is significantly reduced in obese individuals, which partially explains reduced AMPK activity in these individuals.⁹⁹ Importantly, it has been proposed that adiponectin explains many beneficial effects of calorie restriction, including insulin sensitization through activation of AMPK.¹⁰⁰

Ghrelin, a hunger hormone that is secreted from the stomach during calorie restriction, exerts central orexigenic and peripheral metabolic effects that are antagonized by leptin.¹⁰¹ Ghrelin activates AMPK in the hypothalamus by promoting intracellular calcium-induced CaMKK2 activation through the stimulation of food intake.^{101,102} In contrast, ghrelin inhibits AMPK in the adipose tissue and liver by exerting lipogenic and gluconeogenic effects.¹⁰³ A recent study suggested that ghrelin is essential for survival during severe calorie restriction or fasting by maintaining blood glucose levels.¹⁰⁴

Obesity and inflammation

Accumulating data suggest that chronic inflammation is a critical risk factor of modern chronic diseases, including insulin resistance, diabetes and cancer, and that obesity is a risk factor of chronic inflammation. In macrophages and adipose tissue, FFAs or lipid infusion can trigger the proinflammatory response by binding to toll-like receptor 4, which induces insulin resistance.¹⁰⁵ Interestingly, compelling evidence has indicated a negative association between obesity/inflammation and AMPK.¹⁰⁶ Consistently, a recent study showed that reduced AMPK activity was associated with increased inflammation in the visceral adipose tissue and whole-body insulin resistance in morbidly obese individuals.¹⁰⁷ Initial studies suggested that the proinflammatory cytokine $\text{TNF}\alpha$ suppressed AMPK phosphorylation and activate increasing PP2C expression in the skeletal muscles, thus contributing to insulin resistance.¹⁰⁸ Consistently, lipid infusion stimulated the proinflammatory response in the heart by upregulating IL6 and SOSC3 expression, which decreased AMPK phosphorylation and protein levels.¹⁰⁹ A recent study showed that LPS, FFAs and diet-induced obesity downregulated the expression of LKB1 and phosphorylation of the AMPK $\alpha 1$ -subunit, a major isoform of the AMPK α -subunit, in the adipose tissues and macrophages, suggesting that AMPK is suppressed by multiple mechanisms.¹¹⁰ In addition to cytokines and FFAs, resistin is involved in inflammation and insulin resistance. Resistin, which is mainly secreted by macrophages and neutrophils in humans during inflammation, promotes the proinflammatory response and induces insulin resistance.^{111–113} Although

mechanisms underlying these effects of resistin are unclear, it has been suggested that resistin-induced insulin resistance is partially mediated by AMPK inhibition through the proinflammatory signals that induce PP2C or SOSC3 expression.^{112,114,115} Another study showed that anti-inflammatory stimuli induced by $\text{TGF}\beta$ and IL10 activate AMPK in macrophages; however, upstream kinases involved in this activation have not been identified.¹¹⁶ TAK1 is one possible kinase involved in the phosphorylation of AMPK during the anti-inflammatory response; however, this warrants further investigation.

Several studies suggest that AMPK exerts potent anti-inflammatory effects, as summarized in Figure 2. Intensive research using various cell types indicates that AMPK inhibits inflammation by indirectly inhibiting $\text{NF}\kappa\text{B}$, a key regulator of innate immunity and inflammation. Although the mechanisms underlying AMPK-induced inhibition of $\text{NF}\kappa\text{B}$ are not clearly understood, multiple mechanisms that suppress the expression of inflammatory genes, including activation of SIRT1, FOXO and $\text{PGC1}\alpha$, may be involved.¹¹⁷ Interestingly, emerging evidence suggests that AMPK exerts anti-inflammatory effects in immune cells by switching metabolism from glycolysis to mitochondrial oxidative metabolism, such as FAO.¹⁰⁶ Resting lymphocytes depend on mitochondrial oxidative metabolism. However, upon stimulation, metabolism in these cells is switched to glycolysis, which is associated with AMPK inhibition and rapid cell proliferation. Similarly, a recent study showed that switching from proinflammatory M1 macrophages to anti-inflammatory M2 macrophages depends on AMPK and FAO.¹¹⁸ These data suggest that AMPK has a key role in improving inflammation and insulin sensitivity by regulating FAO.

Exercise and muscle contraction

Exercise-induced muscle contraction exerts many beneficial effects on health, including an insulin-sensitizing effect, largely through activation of AMPK. Muscle contraction is an energy-demanding process that greatly increases ATP turnover by over 100-fold, and induces rapid accumulation of ADP and AMP in an intensity-dependent manner.¹¹⁹ In addition, muscle contraction depolarizes T-tubules that induce calcium release from the sarcoplasmic reticulum in muscle cells.¹²⁰ Muscle contraction-induced increases in energy stress and calcium levels promote LKB1- and CaMKK2-mediated AMPK activation, respectively.

Aging

Because metabolism and calorie restriction are well-recognized regulators of aging, several studies on aging have focused on AMPK. Recent studies have shown that AMPK activation in response to various stimuli, such as exercise and muscle contraction, gradually declines during aging.¹²¹ Although mechanisms underlying this have not been elucidated, it is possible that the age-related increase in chronic inflammation levels suppresses AMPK activation in aged tissues.¹²² Importantly, numerous studies have shown that AMPK plays a

crucial role in regulating longevity and calorie restriction-induced lifespan extension in worms, fruit flies, and rodents. Intensive research has identified several key AMPK-regulated pro-longevity pathways, including inhibition of CRTC-1/CREB, NF κ B, and mTORC1 and activation of SIRT1, NRF2, FOXO1, and ULK1, which induce antioxidant defense, anti-inflammation, and autophagy.¹²¹

ROLE OF AMPK IN DIABETES AND CANCER: LESSONS FROM TWO OLD DRUGS

Type-2 diabetes

Type-2 diabetes is a metabolic syndrome caused by insulin resistance that induces hyperglycemia, hyperinsulinemia and hyperlipidemia. Intensive research has shown that prolonged exposure to excessive nutrients is one of the critical risk factors of insulin resistance.⁸⁴ High FFAs can drive insulin resistance through DAG accumulation and PKC activation, which impairs insulin signaling by phosphorylating (IRS)-1/2, the insulin receptor substrate in the skeletal muscles, adipocytes and the liver.^{123,124} In addition, high amino acids (especially BCAAs) and insulin elicit mTORC1 hyperactivity, which impairs insulin signaling through p70S6K-induced IRS-1/2 phosphorylation.^{125,126} mTORC1 hyperactivity also elicits insulin resistance through ER stress, which induces ROS generation and chronic inflammation.^{125,127} Notably, FFAs can induce chronic inflammation both directly by activating TLR4

signaling¹⁰⁵ and indirectly through ER stress.^{128,129} Importantly, all these conditions are associated with reduced AMPK activity (Figure 3). As shown in Figures 2 and 4, AMPK activation improves insulin sensitivity by inhibiting lipogenesis (ACC1, SREBP1c), protein synthesis (mTORC1) and lipolysis (HSL), and by activating FAO (ACC2). These pathways are associated with reduced inhibitory phosphorylation of IRS-1/2, ER stress/ROS, FFAs and chronic inflammation. Indeed, metformin, an indirect activator of AMPK, is the most frequently prescribed antidiabetic drug for type-2 diabetic patients. Therefore, AMPK-activating agents would be beneficial for both preventing and treating patients with type-2 diabetes.

Cancer: initiation and promotion vs progression and metastasis

Tumor suppressor LKB1 functions as an upstream kinase, and mTORC1 functions as a downstream effector of AMPK. Therefore, AMPK activation would be a promising therapeutic strategy because it inhibits mTORC1. However, as extensively discussed,^{2,27,130} the role of AMPK in cancer is complicated, similar to a double-edged sword. Previous studies reported that LKB1 is mutated in 20–30% of patients with cervical and lung cancers. Recent cancer genomic studies reported that several AMPK subunits are frequently overexpressed in cervical and lung cancers.^{131,132} A recent study also showed that

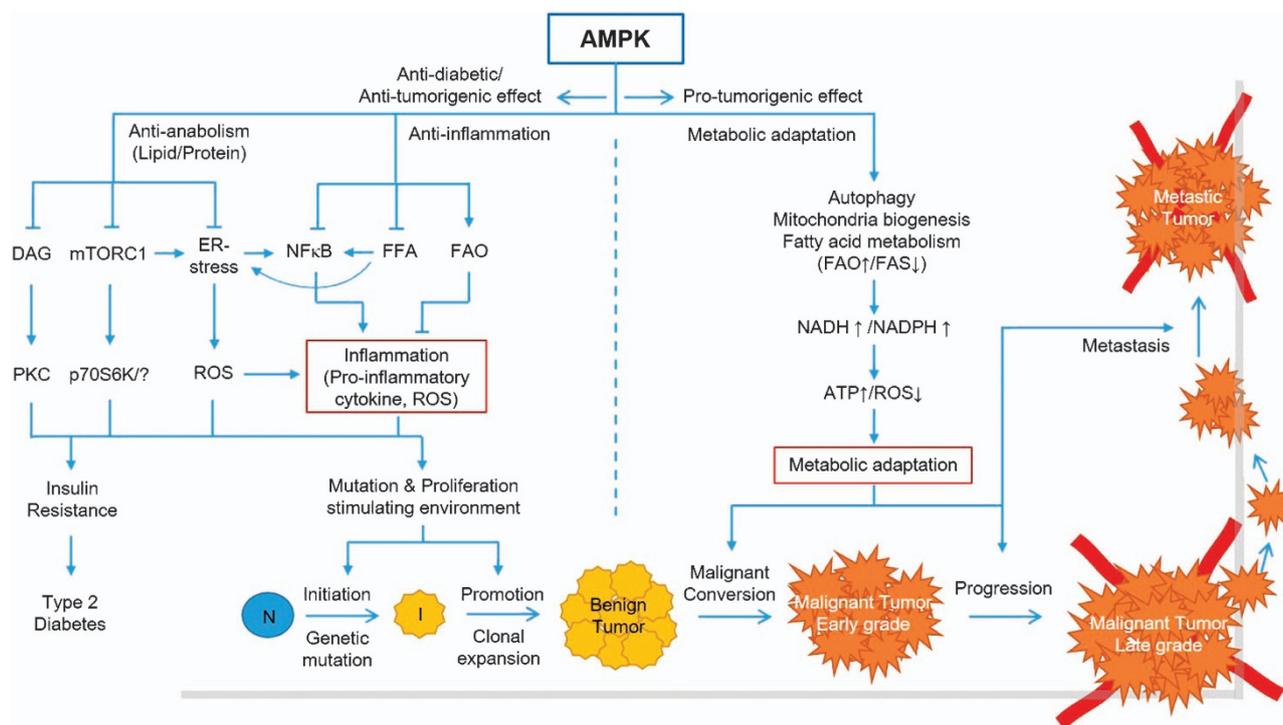


Figure 4 Integrative role of AMPK in diabetes and cancer. This model describes the integrative role of AMPK in diabetes and cancer. AMPK exerts anti-inflammatory effects largely by regulating FA metabolism, NF κ B and ER stress, indicating that AMPK activators can be used for both preventing and treating insulin resistance and diabetes. The anti-inflammatory effects of AMPK also prevent cancer by inhibiting the cancer initiation and promotion stages. However, AMPK activation can also promote malignant conversion, progression and metastasis by enabling metabolic adaptation of tumor cells. Arrow indicates activation, bar-headed line indicates inhibition (see text for details). N, normal cells; I, initiated cells.

MAGE-A3/6 and TRIM28 E3 ubiquitin ligase, which are overexpressed in many cancers, induce cancer-specific AMPK degradation. In contrast, another study showed that SKP2-driven LKB1 ubiquitination in cancer cells increases its activity toward AMPK activation and promotes tumor growth.^{23,46} As shown in Figure 4, one possible explanation for this discrepancy in the role of AMPK in cancer is the timing of modification, mutation, or overexpression of LKB1 or AMPK. The theory of multistep carcinogenesis indicates that the tumor initiation stage, which is characterized by the introduction of DNA mutations in normal chromosomes, favors the formation of a stressful and proinflammatory environment for inducing genetic mutations.¹³³ Inactivation of the LKB1-AMPK pathway during this stage may facilitate both cell growth and proliferation by activating mTORC1 and anabolic pathways, and by introducing genetic mutations through the augmentation of oxidative stress and proinflammatory response, largely through dysregulation of FA metabolism (Figure 4). In fact, this model can be supported by a recent finding that LKB1 deficiency promotes neutrophil recruitment and proinflammatory cytokine production in the lung tumor microenvironment.¹³⁴ After the introduction of multiple genetic mutations and growth of cells into large clones such as benign tumors, the cells need to adapt and survive more severe metabolic and oxidative stress and proinflammatory environments to completely transform into malignant tumor cells (malignant conversion and progression stage). In such cases, activation of the LKB1-AMPK pathway would be beneficial for tumors because it promotes metabolic adaptation. Interestingly, this is also largely achieved by the regulation of FA metabolism, which contributes to the maintenance of NADH and NADPH levels, increases ATP levels and decreases ROS levels (Figure 4).

This view is supported by the findings that LKB1 deletion results in the formation of benign intestinal polyps that are resistant to transformation and that expression of the AMPK α 2-subunit is suppressed in grades I and II human gastric cancers but increased in grades III and IV human gastric cancers.^{135,136} This view also points out the conflicting roles of FA metabolism in different tumor stages because FAO activation and FAS inhibition can prevent early stages but promote late stages of carcinogenesis (Figure 4). However, this model does not explain how tumor cells in the initiation stage that harbor mutations in the gene encoding LKB1 survive malignant conversion and progression, and develop into malignant tumors. This could be explained by the possibility that malignant transformation of tumor cells could be induced by the co-occurrence of complementing genetic mutations or activation of other AMPK-activating pathways involving CaMKK2 or TAK1.^{27,130,137} Therefore, it is necessary to distinguish between the roles of AMPK in cancer depending on the stages of carcinogenesis. Moreover, this suggests that AMPK activation is beneficial for cancer prevention but not for cancer treatment. Rather, AMPK inhibition could be used for treating established cancers by inhibiting stress adaptation and survival.

Lessons from two old drugs: anti-inflammation as a converging point and key mechanism for preventing both diabetes and cancer by AMPK activation

A recent study showed that salicylate, a natural product and *in vivo* metabolite of the anti-inflammatory drug aspirin, directly activates AMPK by binding to its β 1-subunit.¹³⁸ This direct effect on AMPK activation could explain the anti-inflammatory effect of aspirin. Moreover, a previous study found that aspirin reduces circulating FFA and TG levels in obese patients with type-2 diabetes and increases FAO during fasting in healthy humans, which can be explained by the direct effect of aspirin on AMPK activation.^{139,140} Interestingly, aspirin exerts chemopreventive effects and is used for cancer prevention.¹⁴¹ The critical role of AMPK in metabolism and inflammation suggests that aspirin could be effective for treating insulin resistance and diabetes through AMPK activation. In addition, anti-inflammatory effects of metformin by activating AMPK have been reported.¹⁴² Moreover, an epidemiological study showed that the use of metformin in diabetic patients significantly decreased the incidence of various cancers.^{143,144} Notably, chronic inflammation is one of the most critical and common risk factors of cancer and diabetes. Thus, the results of studies on aspirin and metformin suggest that AMPK-induced suppression of chronic inflammation could be a key mechanism by which activation of AMPK can prevent both diabetes and cancer (Figure 4).

CONCLUDING REMARKS

Many studies have demonstrated that AMPK is inhibited in many pathological conditions, such as inflammation, diabetes, aging and cancer, and that activation of AMPK can be beneficial to treat such diseases. Importantly, emerging data using the two old drugs known to activate AMPK suggest that the beneficial effects of AMPK activation can be largely attributed to its anti-inflammatory effects. Notably, the anti-inflammatory role of AMPK is mediated at least in part by the regulation of FA metabolism (FAO \uparrow /FAS \downarrow). However, caution is needed when considering the role of AMPK in cancer because it performs both anti- and pro-tumorigenic roles, depending in part on the regulation of FA metabolism. The pro-tumorigenic role of AMPK involves promotion of metabolic adaptation for cancer cell survival by regulating FA metabolism to maintain ATP and ROS levels during metabolic stress in the tumor microenvironment. Collectively, we propose two converging points, FA metabolism and inflammation, in the mechanisms by which AMPK has a role in diabetes and cancer. First, AMPK could be beneficial for preventing both diabetes and cancer by suppressing inflammation via modulation of FA metabolism. Second, AMPK can promote late stages of carcinogenesis through modulation of FA metabolism in tumor cells to induce metabolic adaptation in a metabolically stressful tumor microenvironment. Thus, AMPK activation is a promising strategy for preventing both diabetes and cancer, whereas AMPK inhibition is a novel therapeutic strategy to treat established cancers.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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