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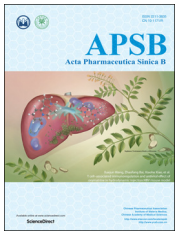

REVIEW

Dissecting the role of AMP-activated protein kinase in human diseases

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Abstract AMP-activated protein kinase (AMPK), known as a sensor and a master of cellular energy balance, integrates various regulatory signals including anabolic and catabolic metabolic processes. Accompanying the application of genetic methods and a plethora of AMPK agonists, rapid progress has identified AMPK as an attractive therapeutic target for several human diseases, such as cancer, type 2 diabetes, atherosclerosis, myocardial ischemia/reperfusion injury and neurodegenerative disease. The role of AMPK in metabolic and energetic modulation both at the intracellular and whole body levels has been reviewed elsewhere. In the present review, we summarize and update the paradoxical role of AMPK implicated in the diseases mentioned above and put forward the challenge encountered. Thus it will be expected to provide important clues for exploring rational methods of intervention in human diseases.

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1. Introduction

Since 1973, AMP-activated protein kinase (AMPK) has been first known as an inhibitory factor of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCR) and acetyl-coenzyme A carboxylase (ACC) in the presence of ATP. The responsible agents were later shown to be kinases and subsequently named as HMGCR kinase and ACC kinase 3, respectively[1,2]. With the progress of successful purification of this kinase, it was not until 1989 when the name AMPK was finally adopted as it can be allosterically regulated by AMP[3]. Besides key regulatory enzymes controlling sterol and fatty acid synthesis, two other enzymes that catalyze important steps in lipolysis and glycogen synthesis (hormone-sensitive triglyceride lipase and glycogen synthase) were subsequently reported as substrates for AMPK[4,5]. The physiological role of AMPK gradually surfaced. In the meantime, the recognition of nucleotides, AMP, ADP and ATP, as allosteric regulators of AMPK activity deepened the understanding of the physiological significance of AMPK[3]. The current view is that AMPK, the cellular energy sensor, is activated following cellular stress like hypoxia, starvation, glucose deprivation or muscle contraction which can increase the ratio of ADP:ATP or AMP:ATP. In order to restore cellular energy balance, the highly active enzyme integrates hormonal and nutrients signals which will promote catabolism (fatty acid oxidation and glycolysis) and inhibit anabolism (fatty acid, cholesterol, triglycerides and protein, etc.). Apart from these canonical functions, AMPK is confirmed to be involved in cell growth, development, longevity and cell polarity[6]. Additional AMPK activating factors include liver kinase B1 (LKB1), calmodulin-dependent protein kinase kinase β (CaMKKβ) and transforming growth factor-β-activated kinase (TAK-1). LKB1 has been proposed to phosphorylate AMPK when the ratio of AMP:ATP is upregulated. However, AMPK can still be excited by CaMKKβ during intracellular Ca^{2+} release or by TAK-1 in response to pro-inflammatory cytokines or apoptosis-inducing agent, even when no change in nucleotides are detected[7–10]. The detailed information regarding structure, activity regulation and pharmacological agonists of AMPK have recently been reviewed[11]. In this article, we will focus on the association between AMPK and a variety of human diseases, including cancer, type 2 diabetes, atherosclerosis, myocardial ischemia/reperfusion injury and neurodegenerative disorder.

2. The role of AMPK in human diseases

2.1. AMPK in cancer

Cancer is fundamentally a disease of tissue growth regulation failure, which can be driven by dysregulation of several cell cycle components. Apart from these alterations, increased catabolic glucose metabolism was observed in proliferating cells. It is a necessity for tumor cells to overcome the significant energy challenge in the initiation of uncontrolled proliferation; otherwise, they die due to energy deficiency. In the mid-1950s, Warburg[12] discovered that tumor cells still survive in the absence of oxygen by glycolysis, and later it was referred as "Warburg effect". The metabolic switch towards the Warburg effect not only supplies the biogenetic source but also confers important metabolic intermediates for cell growth[13]. Presently, this peculiar metabolic shift is universally recognized as a hallmark for tumor cells. Progress in identification of oncogenes and tumor suppressor genes makes the targeting of this metabolic switch a viable new approach for cancer treatments.

It has been proposed that AMPK is closely related to the regulation of the cell cycle because AMPK activation stimulates phosphorylation and activation of tumor suppressor p53, stabilization of cyclin-dependent kinase inhibitor p27 and reduction of key cell cycle regulators like cyclin A and cyclin B1[14–16]. Oncogenic *BRAF* repressing LKB1 and AMPK activity accelerates melanoma cell proliferation[17]. On the other hand, AMPK activation blocks the progression of keratinocyte cell cycle *via* phosphorylation of B-Raf[18]. A recent study revealed that CAMKKβ-induced AMPK activation also induces cell cycle arrest[19]. Apart from the regulatory effect on cell cycle checkpoints, AMPK suppresses the anabolic processes required for rapid cell growth. These processes include mammalian target of rapamycin complex 1 (mTORC1)-dependent protein biosynthesis induced by direct phosphorylation of the tumor suppressor TSC2 and the regulatory-associated protein raptor[20], and *de novo* biosynthesis of fatty acid and cholesterol caused by inactivating ACC1, HMGCR and lipogenic transcription factors sterol regulatory element-binding proteins (SREBPs)[21,22]. More recent genetic researches showed that the growth-suppressive action of AMPK may be mediated by the Hippo pathway[23–25]. Furthermore, Faubert et al.[26,27] provided evidence to demonstrate that AMPK is a negative controller of the Warburg effect using models with genetic deletion of either LKB1 or AMPKα1. AMPK activation promotes mitochondrial biosynthesis and expression of oxidative enzymes and thus attenuates the glycolytic pathway by inhibiting the transcription factor hypoxia-inducible factor 1α (HIF-1α). Genetic ablation of AMPKα1 accelerates Myc-induced lymphomagenesis, suggesting that the absence of AMPK may enhance oncogene activity to boost tumorigenesis[26]. Thus, AMPK can be classified as a metabolic tumor suppressor (Fig. 1).

However, several groups have recently reported that diverse variety of tumor repressors or proto-oncogenes negatively regulate AMPK activity. The absence of the tumor repressor gene folliculin (*FLCN*) in association with Birt-Hogg-Dube syndrome (BHD) confers tumorigenesis through activation of AMPK[28,29]. Likewise, AMPK stimulation was found to be indispensable for the proliferation of astrocytic tumor cells or the growth of experimental human breast cancer tumor[30,31]. Besides, microphthalmia-associated transcription factor (MITF), a melanoma oncoprotein, is regulated by AMPK to maintain cell viability[32]. The requirement of AMPK for prostate cancer progression and colon tumor cell survival has also been recently reported[33,34]. It seems that there are two faces of AMPK in tumorigenesis. In established solid tumors, AMPK activation can provide metabolic adaptive responses to maintain energy supply, although inhibition of AMPK is beneficial in earlier phases of tumor growth. It is worth noting that AMPKα2 has been reported to selectively suppress Ras-induced mouse embryo fibroblasts (MEFs) transformation and reduce the growth of human mammary epithelial cells (HMECs)[35,36]. However, two more recent reviews have pointed out that the *PRKAA1* gene encoding α1 is often amplified whereas the *PRKAA2* gene encoding α2 is more frequently mutated in human cancers[37,38]. Thus, the two catalytic subunit isoforms may play divergent roles in cancer.

In consideration of the aforementioned role of AMPK in tumorigenesis, pharmacological activation of AMPK may exert beneficial effects on cancer. Indeed, pharmacological activation of AMPK by biguanides or A769662 to $Pten^{+/-}$ mice remarkably inhibits

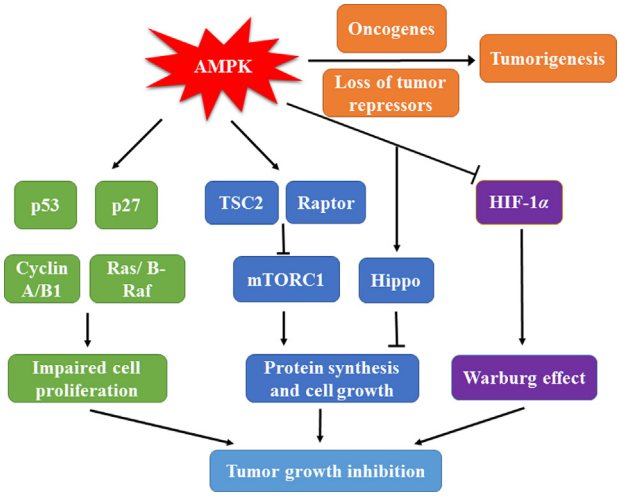

Figure 1 AMP-activated protein kinase (AMPK) plays a double-edged role in cancer. AMPK stimulation promotes activation of tumor repressor p53, increases cyclin-dependent kinase inhibitor p27, decreases cyclin A and cyclin B1, and suppresses ras and B-raf signaling pathway, resulting in cell cycle arrest and impaired cell proliferation. AMPK can induce phosphorylation of TSC2 and raptor to suppress mTOCR1 dependent protein synthesis and activate Hippo pathway to inhibit cell growth. The reduced HIF-1α transcription activity mediated by AMPK activation leads to the block of Warburg effect and the decreased energy supply, contributing to inhibition of tumor cell growth in further. Thus, AMPK may exert its anticancer activities through multiple approaches mentioned above. However, AMPK may also cooperate with either oncogenes or loss of tumor repressors to promote tumorigenesis.

tumorigenesis[39]. Subsequently, numerous studies have obtained similar results showing that AMPK agonists can inhibit cancer cell growth and proliferation[40–42]. In clinic, a significant reduction in the incidence of cancers in subjects taking metformin compared with other antidiabetic drugs was found by meta-analysis[43]. However, several groups have recently challenged the role of AMPK in the protective effect of these compounds on tumorigenesis. They pointed out that A769662, widely acknowledged as direct AMPK agonist, may actually accelerate tumor cell proliferation in response to metabolic stress. Additionally, those indirect AMPK agonists prevent tumor cell proliferation and mTOR activity in an AMPK-independent manner[44–46]. Biguanides, a group of classic anti-diabetic drugs, was later recognized as potential anti-cancer agents. In fact, they are inhibitors of the mitochondrial respiratory chain and have recently been found to preferentially kill various cancer stem cells, which are dependent on mitochondrial metabolism[47,48]. In the context of inhibition of proliferation, the upregulation of AMPK activity occurs as an adaptive response to protect cells from the toxicity of biguanides since the mortality increases in cells without LKB1, a critical upstream kinase of AMPK and once known as a tumor repressor[49]. Further, in the mouse model of colon carcinoma or non-small cell lung cancer with a defective LKB1/AMPK pathway, the rate of tumor growth declines following treatment with biguanides[50,51]. Thus, it would be promising to combine biguanides with AMPK inhibitors in the treatment of established solid tumors.

2.2. *AMPK in type 2 diabetes*

Type 2 diabetes (formerly named noninsulin-dependent diabetes mellitus or adult-onset diabetes) is a metabolic disorder characterized by hyperglycemia and abnormal lipid metabolism in the context of decreased insulin sensitivity of peripheral tissue and inadequate insulin secretion by islet beta cells. It has been agreed that overnutrition, inactivity and consequential obesity are the primary cause of type 2 diabetes in genetically predisposed individuals. Regular exercise and proper dietary are thought to be first steps to manage the disease. The beneficial effects of exercise may be at least partly mediated by AMPK activation, consistent with a critical role of AMPK in regulating glucose metabolism[52].

The above mentioned insulin-insensitive peripheral tissues mainly consist of skeletal muscle, liver and fat. Among them, skeletal muscle constitutes around 80% of insulin-stimulated glucose disposal[53]. It has been reported that AMPK activation by chronic administration of metformin enhances insulin-stimulated glucose uptake in mouse soleus muscle[54]. One explanation may be that AMPK activation enhances insulin receptor substrate 1 (IRS-1) Ser789 phosphorylation and subsequent phosphoinositide 3 kinase/protein kinase B (PI3K/PKB) signaling pathway[55,56]. However, *in vivo* evidence to support this is lacking, since either AMPKβ1β2 muscle knockout mice or mice over-expressing AMPKα2 kinase-dead (KD) in muscle have normal insulin-stimulated glucose transport[57,58]. The relationship between AMPK and insulin-independent stimulation of glucose uptake has attracted significant interest. AMPK agonists can stimulate glucose uptake in resting skeletal muscle, and genetic techniques confirmed that these effects are indeed mediated by AMPK[59–61]. Moreover, it has also been found that AMPKα2β2γ3 heterotrimer is mainly activated in skeletal muscle[52]. Although there are significant contradictory reports regarding the role of AMPK in exercise-induced glucose transport, results in muscle-specific AMPKβ1/AMPKβ2 double knockout mice have argued convincingly that AMPK is implicated in glucose transport in response to exercise or muscle contraction[57]. When AMPK is activated by exercise or contraction, both Rab GTPase-activating protein TBC1D4 (also known as AS160) and TBC1D1 are phosphorylated and inactivated. Nevertheless, TBC1D1 plays a more pivotal role. The phosphorylated form can recruit scaffolding protein 14-3-3 and allow GLUT4 storage vesicles transport to plasma membrane[62,63] (Fig. 2).

Apart from impaired glucose uptake in skeletal muscle, the excessive release of glucose into the circulation by liver is another

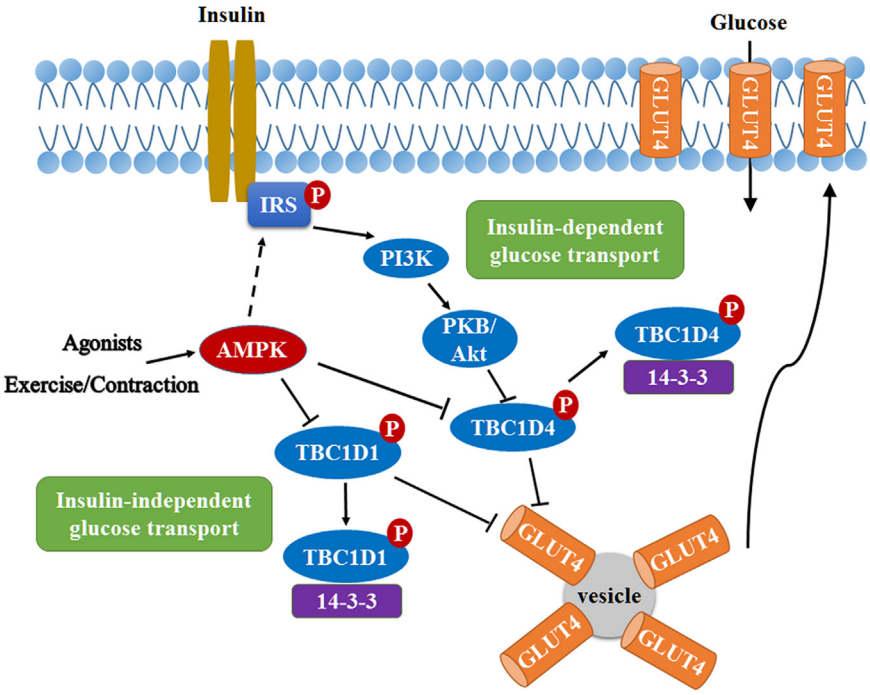

Figure 2 Regulation of glucose uptake in muscle by AMPK. AMPK activation by exercise or muscle contraction or agonists can phosphorylate Rab GTPases TBC1D4 and TBC1D1, increase 14-3-3 binding and subsequent dissociation from GLUT4, which promotes glucose uptake through increasing translocation of GLUT4 to plasma membranes. On the other hand, the insulin-mediated glucose uptake includes insulin receptor and insulin receptor substrate phosphorylation, PI3K/Akt activation and then phosphorylation of TBC1D4. AMPK may participate in the phosphorylation of insulin receptor substrate-1 *in vitro* but there is few *in vivo* evidence to support this.

contributor to hyperglycemia. Dysregulated hepatic glucose production is mainly caused by abnormal gluconeogenesis and elevated plasma glucagon levels. Metformin, a widely used anti-diabetic agent, was first found to inhibit gluconeogenesis through the signaling pathway of LKB1[64]. However, whether AMPK plays a key role in the reduction of hepatic glucose output by biguanides is quite controversial. Foretz et al.[65] showed that metformin suppresses gluconeogenesis *via* a decrease in hepatic energy state independent of LKB1/AMPK pathway by genetic loss-of-function experiments. Subsequently, other groups also found that the inhibition of glucose output by metformin is attributed to the reduced hepatic glucagon signaling and declining mitochondrial glycerophosphate dehydrogenase activity[66,67]. Conversely, Fullerton et al.[68] reported that metformin-induced AMPK activation and ACC phosphorylation play crucial roles in lipid-induced insulin resistance. It seems that the acute effect of metformin on hepatic glucose output may be AMPK-independent, whereas the longer-term effects (which are probably more relevant to therapy of humans with metformin) are AMPK-dependent. Subsequently, Cao et al.[69] suggested that a low concentration of metformin inhibited gluconeogenic gene expression *via* AMPK without increasing the AMP/ATP ratio in primary hepatocytes. Furthermore, the increase in the net phosphorylation of AMPK Thr172 is caused by metformin-mediated increment in formation of the AMPK complex[70]. More recently, a small-molecule AMPK activator 991 was demonstrated to antagonize hepatic glucagon signaling *via* AMPK-induced cyclic nucleotide phosphodiesterase 4B (PDE4B) activation[71].

Adipose tissue is the main resource for plasma free fatty acids (FFAs) and abundant FFA accumulation in skeletal muscle, liver and adipocytes can drive insulin resistance *via* diacylglycerol (DAG) accumulation and protein kinase C (PKC) activation[72,73]. AMPK activation in adipocytes inhibits lipogenesis due to increased phosphorylation of ACC and decreased expression of lipogenic genes including stearoyl-CoA desaturase 1 (SCD1), fatty acid synthase (FAS) and ACC1, which are under control of transcription factor SREBP-1c[74,75]. On the other hand, the inactivation of ACC contributes to decreased malonyl-CoA and thus the attenuated inhibition of carnitine palmitoyltransferase 1 (CPT1), a critical enzyme for fatty acid oxidation in mitochondria. Although acute treatment of AICAR in adipocytes was found to attenuate fatty acid oxidation, which is associated with reduced fatty acid uptake[76], chronic activation of AMPK-stimulated fatty acid oxidation and mitochondrial biogenesis[74,77]. The role of AMPK in lipolysis is paradoxical. Exercise was reported to promote lipolysis in an AMPK-dependent fashion stimulated by adrenaline in adipocytes[78]. The pro-lipolytic action of AMPK was suggested to be closely associated with increased ATGL phosphorylation[79]. In contrast, mice deficient in AMPKα1 revealed a phenotype of smaller adipocytes with increased lipolysis. The anti-lipolytic effect of AMPK was demonstrated to act on hormone-sensitive lipase (HSL) by blocking its translocation to the lipid droplet[80]. It seems that acute activation of AMPK suppresses adipose lipolysis and thereby decreasing serum fatty acid concentration, whereas prolonged activation promotes lipolysis[74]. A recent study demonstrated that nicotine acts on adipose tissue to accelerate lipolysis and induce insulin resistance through activating AMPKα2[81]. However, the overall impact of AMPK on lipolysis still remains controversial.

The fourth contributor to Type 2 diabetes is the decline in numbers of normally functioning islet β cells, which is concurrent with dysregulated glucose-induced insulin secretion. Granot and other groups[82,83] first showed that genetic deletion of LKB1 in pancreatic beta cells dramatically increased insulin secretion in response to glucose and improved glucose tolerance; dramatic

changes in β cell mass and polarity were also seen *in vivo*. Subsequently, two groups independently reported that LKB1 and AMPK may play different roles in the control of insulin secretion from islet β cells[84,85]. In these studies, AMPK deficiency both in pancreatic β cell and hypothalamic neurons displayed defective insulin secretion and glucose-intolerance. Recently, Kone and his colleagues[86] reconfirmed the above results through developing new models without AMPK deletion in the brain. One possible mechanism involves promotion of AMPK-dependent K_{ATP} channel trafficking, alleviation of endoplasmic reticulum stress and reduction of lipid accumulation *via* autophagy stimulation[87–89]. Thus, AMPK activation seems to have beneficial effect on islet β cells. Indeed, administration of AICAR protects against glucolipotoxicity-induced impaired β-cell function[90]. However, mice with the Arg299Gln γ2-specific mutation develop dysregulated β-cell function and obesity due to sustained activation of AMPK throughout all tissues[91].

Any AMPK activator that crosses the blood–brain barrier would be likely to have adverse effects on food intake because hypothalamic AMPK plays a critical role in the regulation of feeding behavior. This process is suggested closely associated with the expression of orexigenic neuropeptide Y (NPY)/agouti-related protein (AgRP) and anorexigenic proopiomelanocortin-α (POMC)[92]. Either pharmacological activation or expressing constitutively active AMPK in hypothalamus increased food intake, whereas expressing dominant-negative (DN) AMPK in hypothalamus decreased the expression of NPY and AgRP[93,94]. Claret et al.[95] reported that mice with AMPKα2 deletion in proopiomelanocortin (POMC)-expressing neurons develop obesity due to increased food intake and decreased energy expenditure. On the contrary, mice with AMPKα2 defective in agouti-related protein (AgRP)-expressing neurons maintain their lean phenotype, suggesting there is a close relationship between AMPK and the activation of these neurons. A recent study pointed out that hypothalamic AMPK regulates neuropeptide expression through induction of autophagy[96].

2.3. AMPK in atherosclerosis

Atherosclerosis is a slow, progressive disease with accumulated cholesterol, triglyceride, immune cells and fibrin in the intima of coronary and larger arteries, which constitutes plaque. The plaque may gradually plug these arteries causing many other cardiovascular diseases including myocardial infarction and stroke. Solid evidence implicates AMPK in atherosclerosis through modulation of macrophage cholesterol homeostasis, inflammation and vascular dysfunction.

Imbalance of macrophage cholesterol homeostasis is of great importance to atherosclerotic progression. This is mostly because plaque formation requires monocyte infiltration, resulting in generation of vast proinflammatory factors and chemokines, and further development into atherogenic foam cells caused by excessive uptake of modified low-density lipoprotein (LDL) particles. AMPK has been shown to prevent cholesterol accumulation in macrophages through promoting cholesterol efflux to high-density lipoprotein (HDL), causing a marked decrease in atherosclerotic plaque in *ApoE*$^{-/-}$ mice[97,98]. The beneficial effect of AMPK may be related to the upregulation of ATP-binding cassette sub-family G member 1 (*ABCG1*) and ATP-binding cassette transporter A1 (*ABCA1*) gene expression accompanying with increased liver X receptor α (LXRα) expression (Fig. 3). Recently, our results also found out that AMPK predominates in macrophage uptake of cholesterol mediated by oxidized LDL (oxLDL) through downregulation of lectin like oxidized low-density lipoprotein receptor 1 (LOX-1) expression[99]. Similar results were obtained by several other groups with AICAR or berberine[100,101]. The underlying mechanism is probably the decreased

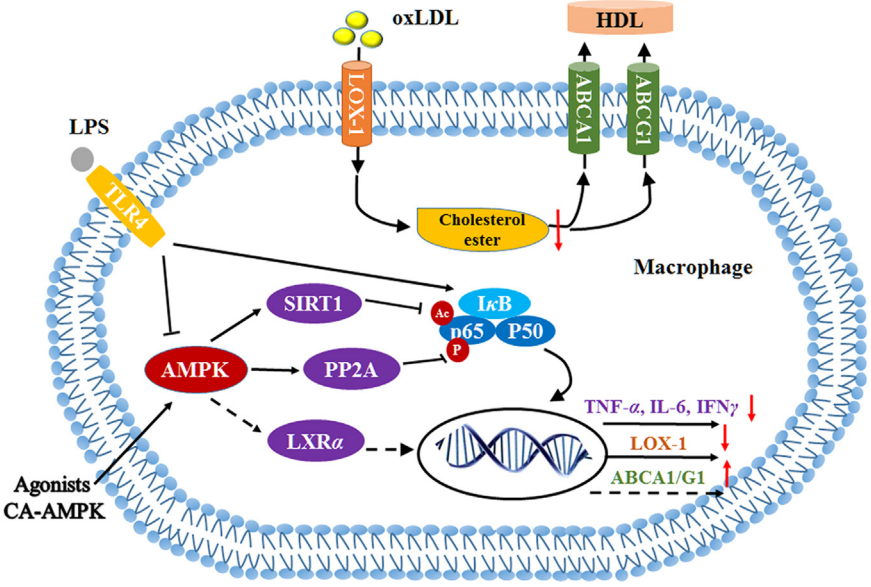

Figure 3 AMPK participates in regulation of macrophage cholesterol homeostasis and lipopolysaccharide (LPS)-stimulated production of inflammation factors. AMPK activation by various agonists (A769662, salicylic acid and AICAR, etc.) or overexpressing constitutively active AMPK upregulates the expression of cholesterol transporter ABCA1 or ABCG1, which may be related to increased expression of nuclear transcription factor LXRα, resulting in accumulated cholesterol efflux to mature HDL. In the meantime, increased PP2A activity stimulated by AMPK causes p65 dephosphorylation and subsequent decreased expression of scavenger receptor LOX-1, resulting in reduced oxLDL uptake and cholesterol accumulation in further. In addition, LPS stimulation can downregulate AMPK activity in macrophage. AMPK activation by pharmacological agonists blocks LPS-stimulated increase of secretary proinflammatory factors by SIRT1-mediated deacetylation of p65.

Ser536 phosphorylation of nuclear transcription factor NF-κB p65 induced by enhanced protein phosphatase 2A (PP2A) activity[99] (Fig. 3). The PP2A B56γ subunit can be directly phosphorylated at Ser298 and Ser336 by AMPK[102]. Nevertheless, recent studies from Zou's group demonstrated that there was no difference between macrophage-specific AMPK deficiency in $ApoE^{-/-}$ mice and corresponding $ApoE^{-/-}$ mice in western diet–induced atherosclerotic plaque formation and plaque instability, which robustly questions the role of macrophage AMPK in atherosclerosis[103,104].

Simultaneously, AMPK seems to participate in inflammatory cytokine release in macrophages, since reduced AMPK activity was found in lipopolysaccharide (LPS)-stimulated macrophages[105]. Accordingly, either activating AMPK in macrophages by pharmacological agonists or constitutive expression of active AMPK attenuates LPS-induced proinflammatory factors, whereas the level of anti-inflammatory cytokine increases, which may be mediated by the reduced acetylation and transcription activity of NF-κB induced by Sirtuin 1 (SIRT1)[106–108] (Fig. 3). Moreover, AMPK-mediated activation of nucleotide-binding domain and leucine-rich repeat containing protein 3 (NLRP3) inflammasome was demonstrated to be involved in both saturated fatty acid–induced inflammation in macrophages and the anti-inflammatory effect of monounsaturated fatty acid[109–110]. Likewise, the NLRP3 inflammasome can also be activated by crystalline cholesterol, an endogenous risk in atherosclerotic progression[111]. However, whether AMPK can regulate this process remains to be addressed.

Vascular dysfunction is acknowledged as an early stage in atherosclerosis and is mainly caused by infiltration of immune cells in the vascular wall, inflammation, oxidative stress, impaired NO bioavailability and endothelial cell apoptosis. All these contributors may be concurrent with a reduction of AMPK activity in aortic endothelium[112]. Consequently, activating vascular AMPK, then altering all above contributors, is probably an effective way to maintain cardiovascular health. It has been demonstrated that expressing constitutively active AMPK in cultured human aortic endothelial cells inhibit TNFα-stimulated leukocyte adhesion associated with reduced monocyte chemotactic protein 1 (MCP-1) secretion[113]. AMPK activation by adiponectin reverses palmitate-induced ROS production and following mitogen-activated protein kinase p38-mediated apoptosis in endothelial cells[114]. Metformin was found to reduce oxidative stress, increase NO bioavailability and restore endothelial function through activation of AMPK/peroxisome proliferator–activated receptor δ (PPARδ) pathway[115]. Several other direct or indirect AMPK agonists were also verified to enhance vascular function in succession[116]. In addition, activation of AMPK promotes vasorelaxation in an endothelium and NO-independent manner, suggesting a direct effect of AMPK on vascular smooth muscle cells (VSMC) apart from endothelium[117]. Recently, two papers from Zou's laboratory were published in succession which revealed that specific AMPKα1 knockout in mouse VSMC promoted western diet–induced aortic calcification while specific AMPKα2 deletion in mouse VSMC induced VSMC phenotypic switching and therefore affected atherosclerotic plaque stability[103,104].

2.4. *AMPK in myocardial ischemia/reperfusion injury*

Myocardial ischemia is mainly caused by an interruption in the coronary blood supply, resulting in detrimental effects such as cardiomyocyte death and cardiac dysfunction. Prompt reperfusion restores myocardial blood flow and oxygen supply, but the process of reperfusion brings about myocardial injury as well. The combination of injury incurred during acute myocardial ischemia and reperfusion following ischemia is therefore named ischemia/reperfusion injury. During ischemia, deficiency of oxygen results in increasing anaerobic metabolism, that is increasing glycolytic pathway instead of oxidative phosphorylation[118]. Although reperfusion of the myocardium can temporally maintain cardiomyocyte viability, metabolic alteration happens since glucose oxidation recovers much slower than fatty acid (FA) oxidation[119]. These alterations, including increased lactate production, proton generation and decreased intracellular pH, can destroy cardiac efficiency and function[120–122].

It has been suggested that AMPK is activated during ischemia and reperfusion due to the depletion of ATP and the increased activity of AMPK kinases[123]. AMPK facilitates the delivery of fatty acids *via* increased cardiac lipoprotein lipase (LPL) activity and increased CD36 expression in membrane[124,125]. The availability of fatty acids accelerates rates of their utilization, which is also promoted by AMPK activation due to decreased ACC activity, thus reducing malonyl-CoA levels. Enhanced fatty acid oxidation during ischemia and reperfusion suppresses glucose oxidative phosphorylation, which is detrimental to the ischemic myocardium[122]. Metabolic and pharmacological activation of AMPK promotes GLUT4 translocation to the sarcolemmal membrane[62], whereas glucose uptake remains unchanged despite the AMPK activation after ischemia[126]. AMPK can also increase phosphofructokinase-2 phosphorylation, resulting in enhanced glycolysis during ischemia[127].

It seems that ischemia-induced AMPK activation is harmful to the heart due to the stimulation of fatty acid oxidation or the glycolytic pathway instead of glucose oxidation. Chang et al.[128] suggested that berberine exerted cardioprotective effects by depressing AMPK activity in ischemic areas of rat heart, whereas AMPK was activated in the non-ischemic areas. In fact, AMPK is more like an adaptive response to satisfy the need of ischemic myocardium for ATP and protect the heart from oxygen deprivation. Brief periods of ischemia preconditioning have been confirmed to provide significant protection to the heart from ischemic injury. AMPK is shown to be involved in the setting of ischemic preconditioning and promotes glucose uptake[129]. Considerable evidence demonstrates that AMPK stimulation prevents post-ischemic cardiac dysfunction and cell apoptosis upon reperfusion. For example, when isolated hearts from an AMPKα2 kinase dead transgenic mice suffered ischemia attack, cardiac function declined, implying that AMPK activation is necessary for the heart to withstand an ischemia insult[130]. Besides, treatment with A769662 prior or during ischemia diminishes the cardiac infarct size and decreases myocardial necrosis in an animal model[131,132]. It has been suggested that adiponectin prevents the heart from ischemia/reperfusion injury in an AMPK and cyclooxygenase 2 (COX-2) dependent manner[133]. Recently , some physiologically secreted factors and stress-inducible proteins, such as follistatin-like 1, C1q/TNF related protein 9, antithrombin, omentin and sestrin 2, have also been discovered to benefit the heart from ischemia/reperfusion injury by stimulating cardiac AMPK[134-138].

2.5. *AMPK in neurodegenerative disorder*

Life-threatening, incurable diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) are well-known examples of neurodegenerative disorder, defined as a progressive loss of neuronal structure and function. AD is the most familiar dementia, featuring senile plaques and intracellular neurofibrillary tangles, primarily caused by accumulation of misfolding Aβ peptides and tau hyperphosphorylation in cortical

and hippocampal brain regions[139]. PD is generally recognized as neurodegenerative motor impairment. PD pathology includes a lack of dopaminergic neurons in the substantia nigra and clumps of α-synuclein, also called Lewy body. One of the main contributors to PD is mitochondrial dysfunction associated with a mutated gene[140]. HD is an age-related disorder involved in both movement and cognizance, induced by CAG triplet repeat expansion in exon 1 of the *Htt* gene. The major pathological change is the severe decrease of neurons used to synthesize enkephalin and γ-amino-butyric acid (GABA)[141].

In mouse models of AD, PD or HD, pathological activation of brain AMPK has been demonstrated[141–143], consistent with the hypothesis that AMPK plays a key role in the development of senile dementia. However, many other reports have shown that AMPK seems to be a double-edged sword, either aggravating or alleviating neurodegeneration under different circumstances.

In AD, it is well established that AMPK is an important modulator of Aβ generation. Resveratrol prevents Aβ accumulation through promotion of autophagy induced by AMPK activation and mTOR inhibition[144]. Similar results were acquired with another AMPK agonist AICAR[145]. Such effects may be associated with changes of neuronal cholesterol and sphingomyelin contents and APP distribution in membrane[139]. In addition, Greco et al.[146] identified that AMPK activation is related to the effect of leptin in neuronal cells, including reduction of tau phosphorylation which could lead to neurofibrillary tangles. On the contrary, some studies pointed out that AMPK inhibition by compound C could improve long-term potentiation (LTP) and alleviate impairments induced by amyloid beta[147]. Moreover, chronic treatment with metformin was reported to have beneficial effects in females but to enhance memory dysfunction in males, suggesting that the effect of AMPK activation on neuronal cells may be gender-dependent[148].

In PD, AMPK activation mitigates dopaminergic dysfunction in *Drosophila* models[149]. The neuroprotective effect of ghrelin, a gut hormone, during calorie restriction was mediated by the AMPK signaling pathway[150]. On the contrary, inhibition of AMPK by compound C in SH-SY5Y cells supplemented with MPP resulted in neuronal cell death[143]. It was suggested that AMPK cooperates with parkin, an important modulator to maintain mitochondrial homeostasis, which explains why AMPK activation may be beneficial in PD[151]. However, Kim et al.[152] reported that PARP triggers the degeneration of dopaminergic neurons through activation of AMPK as well, adding the complexity of the roles for AMPK in PD.

It was especially highlighted in HD that overactivation of AMPK by high-dose AICAR in striatal neurons facilitated neuronal loss and formation of Htt aggregates[141]. The underlying mechanism may be related to AMPK as sensor of oxidative stress to elicit neuronal atrophy[153]. However, Vazquez-Manrique et al.[154] suggested that treatment of HD with metformin may be protective. A potential explanation of the controversial results was that AMPK activation may be beneficial in the onset of HD.

3. Conclusions and perspectives

AMPK has evolved to sense diversified energy and metabolic stress such as produced by hormones, cytokines, growth factors, sheer stress, hypoxia, some xenobiotics, etc. Simultaneously, it may help organisms to survive sudden or chronic stress through regulating a great assay of downstream targets involving glucose, fatty acids, cholesterol and amino acid metabolism, glucose transport, mitochondrial function, cell growth, etc. The evidence to date indicates that AMPK is implicated in various human diseases. In the early stage, AMPK was suggested as a prime drug target for type 2 diabetes since several agonists displayed dramatic therapeutic potential in this disease. Later, it was exciting to discover the beneficial effect of metformin on cancer. Thus, the role of AMPK in tumorigenesis draws significant attention. In addition, other human diseases like atherosclerosis, myocardial ischemia/reperfusion injury and neurodegenerative disorder are all closely related to metabolism and inflammation processes in which AMPK has been identified to be a critical contributor. However, further studies are required to investigate the underlying molecular mechanisms. Noteworthy, a paradoxical role of AMPK was observed in these diseases. There are four possible reasons. First, indirect AMPK agonists such as AICAR and biguandes were used in most studies to demonstrate the beneficial effects of AMPK activation, even though the direct agonist A769662 may exert its effects in an AMPK-independent way under certain circumstances. Second, it should be noted that LKB1 has at least 13 other substrates other than AMPK[155]. When referring to the knockout of LKB1 in mice, some observed phenomena may be mediated by other targets but not AMPK. Third, the widely used AMPK inhibitor, compound C, has also been reported to be highly nonspecific[156]. Fourth, AMPK is such a sensitive sensor to various stresses and its multi-subunit structure and complicated activity regulatory mechanism bring complexity to understanding the role of AMPK in these diseases. Therefore, appropriate genetic models and tissue- or isomer-specifically direct AMPK agonists are desperately needed to differentiate the functions of AMPK in these human diseases.

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